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CONTENTS

Phytotoxic Blood Sera in Relation ..... A...
Antibacterial Activity of Soluble Extracts of Cultivated ...
Hu...
The Isolation of Scopoletin as Zinc-Fluorescing Compound ...
Roots ..... Richard H. Goodwin and Frederick ...
Notes on the Cytology of Some Lycopods ..... Dou...
Postglacial Forests in West Central Alberta, Canada
Ho...
Note on the Section Microsporites of Agrostis ..... Al...
New Species of Cyperaceae from British Guiana and Su...
Cl...
Nowakowskiella crassa Sp. Nov., Cladochytrium ...
and Other Polycystic Chytrids from Maryland
Jo...
Torreya .....
Vernacular Names for Shrubs ... on Buried C...
Secaucus, N. J. ... Plants of the Cl...
Index to American Botanical Literature .....

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BULLETIN OF THE TORREY BOTANICAL CLUB
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PHYTOTOXIC BLOOD SERA IN MEDICINE

DAVID I. MACHT

The present report is an admirable illustration of the advantages accruing both to pure research and to practical medicine from a collaboration between the various fundamental physical, chemical, and biological sciences with the basic medical sciences of physiology, pharmacology, and biochemistry.

Twenty-five years ago the present writer, who was at that time a lecturer in pharmacology at the Johns Hopkins Medical School and associated with the father of American pharmacologists, the famous Professor John J. Abel, conceived the idea of making a comparative study in regard to the action of drugs and chemicals between living plant protoplasm and living animal protoplasm. By pharmacology as it is taught in most of our medical schools is meant of course a study of the effects of drugs, chemicals, and poisons on living animal protoplasm, animal tissue, or whole animal organisms. In other words, it is strictly speaking *zoo*pharmacology. However, just as there are two kinds of physiologies, namely, *zoo*physiology or animal physiology and *phyto*physiology or plant physiology, so the author thought to develop a new branch of biology to which he gave the name of *phyto*pharmacology or the study of drugs and chemicals on living plant protoplasm, plant tissues, or whole plants (1, 2).

The effects of drugs and poisons on plants may be studied in many ways. The plant physiologist may study the effects of chemicals on the germination of seeds and respiration of seeds, on the growth of roots and stems and flowers, on the geotropic and heliotropic properties of plants, on the growth of yeasts and fungi, on the behavior of chloroblasts, on protoplasmic streaming, on the vital processes of phytosynthesis, on oxidation and reduction phenomena, etc. In order to become familiar with plant physiological methods, the writer spent a year or more in the laboratories of the late Professor Burton E. Livingston of the Johns Hopkins University, who at that time was one of the leading plant physiologists in this country. In this way various methods of study were learned and selected as practicable for application to medical work.

The most practical and fruitful approach to phytopharmacological problems was found to be in pursuing quantitative phytopharmacological studies on the root growth of *Lupinus albus* seedlings under standardized conditions of light, temperature, humidity, and many other ecological factors. These methods have been described in full in various publications

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by the writer and his collaborators. It was found very early by means of special phytopharmacological techniques that living plants responded often very differently to drugs and chemical agents from the way the same drugs or chemicals affected living animals. Thus for instance, by means of living plant test-objects one could easily distinguish between solutions of organic and inorganic mercury compounds. Again, by means of such very sensitive plant physiological test-objects, one could differentiate even between stereoisomers. Table 1 illustrates well how sensitive seedlings of *Lupinus*

TABLE 1. *Growth of Lupinus albus Seedlings in Solutions of Some Isomers.*

Drug or Chemical	Optic Variety	Concentration in Shive	Phytotoxic Index per cent
Quinine Sulfate	Levo-rotatory	1: 2000	67
Quinine Sulfate	Levo-rotatory	1: 4000	71
Quinidine Sulfate	Dextro-rotatory	1: 2000	90
Quinidine Sulfate	Dextro-rotatory	1: 4000	94
Cinchonidine Sulfate	Levo-rotatory	1: 1000	69
Cinchonine Sulfate	Dextro-rotatory	1: 1000	91
Leucine (alpha-amino-iso-butyl acetic acid)	Levo-rotatory	1: 100	81
Leucine (alpha-amino-iso-butyl acetic acid)	Racemic	1: 100	102
Camphor	Racemic	1: 10000	53
Camphor	Dextro-rotatory	1: 10000	62
Ortho-saligenin	.....	1: 5000	27
Meta-saligenin	.....	1: 5000	62
Para-saligenin	.....	1: 5000	81
Brom-ortho-saligenin	.....	1: 5000	22
Brom-meta-saligenin	.....	1: 5000	41
Brom-para-saligenin	.....	1: 5000	71

albus are to various chemical compounds dissolved in plant-physiological solution. Here we see also the difference in phytotoxicity produced by isomeric organic compounds. Not only is there a difference in toxicity exhibited by the two sets of *structural* isomeric compounds, the saligenins and brom-saligenins, the formulae of which are subjoined (fig. 1), but also by stereoisomers such as those of quinine, cinchonine, leucine, and camphor. Thus for instance, the stereoisomers of quinine, nicotine, adrenalin, and camphor exerted different degrees of inhibition on the root growth of *Lupinus albus* seedlings. The most striking finding, however, was the marked difference between drugs or chemicals derived from the animal world and drugs and chemicals derived from the plant world. Zoogenetic chemicals were found to be much more toxic for living plant protoplasm

than phytogenetic substances. On the other hand, phytogenetic drugs were in general much more poisonous for animal protoplasm than for plant protoplasm.

A single illustration will be very enlightening. Epinephrine or adrenalin is a drug derived from the superarenal glands of animals. It was found to be a very powerful zoopharmacological agent, as is generally known, but it is not generally known that when tested on living plant protoplasm epinephrine is even more toxic for the root growth of *Lupinus albus* seedlings. Vice versa, the drug known as ephedrine, sometimes called "vegetable adrenalin," which is substance derived from the plant world but possessing certain pharmacological properties similar to those of adrenalin

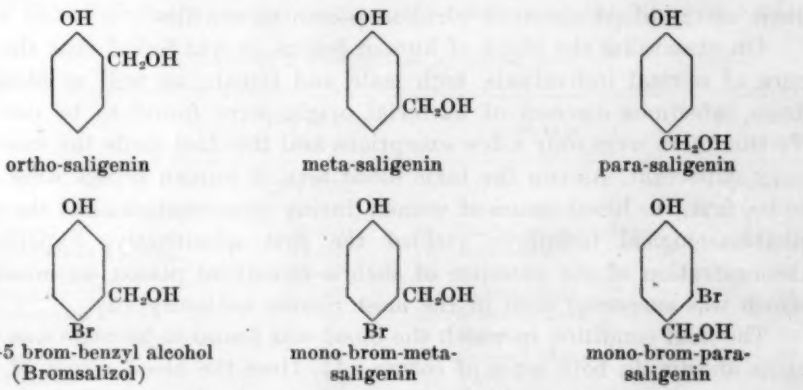

FIG. 1. Chemical structure of certain isomers.

for animals, was found to be much more toxic for animal tissues than for plant tissues.

The most important phytopharmacological findings made by the author and his associates, however, belong strictly to the medical field and are concerned with the biological properties of blood and blood serum. By employing his special technique the author was successful in demonstrating for the first time certain toxic substances in the blood of animals, including man, which could not heretofore be demonstrated either by zoopharmacological methods or even by ordinary chemical and physiological experiments.

Before describing briefly some of these findings with blood, we must define the term often employed by the writer, namely, index of growth. By the index of growth is meant the ratio of the root growth of a series of *Lupinus albus* seedlings grown in plant physiological hydroponic solutions to the growth of seedlings from exactly the same crop in exactly the same plant physiological solutions to which a small percentage (usually 1

per cent) of blood serum or other chemical or drug has been added. In other words,

$$\text{Index of growth} = \frac{X}{N} \times 100$$

It was found on examining the blood sera of all kinds of animals, both cold-blooded and warm-blooded, that the phytotoxic index of a 1 per cent solution usually ranges between 70 and 75 per cent. The only exception to this rule is the blood serum of reptiles (snakes, alligators, lizards, turtles, gila monsters, etc.) which was found to be more toxic than that of other animals. In fact, it was found that the blood serum of so-called non-venomous snakes, or snakes which possess no poison glands, was just as toxic as the blood serum of virulent poisonous reptiles.

On examining the blood of human beings, it was found that the blood sera of normal individuals, both male and female, as well as blood sera from infectious diseases of bacterial origin were found to be non-toxic. To this there were only a few exceptions and this fact made the exceptions very important. Among the toxic blood sera of human beings were found to be, first, the blood serum of women during menstruation; and the phytopharmacological technique yielded the first quantitative experimental demonstration of the presence of such a menstrual poison, or *menotoxin*, which was suspected even in the most remote antiquity (3).

The next condition in which the blood was found to be toxic was pernicious anemia, in both sexes of course (4). Here the blood serum of pernicious anemia patients yielded a very toxic index of growth in contrast to the sera of all other kinds of anemias and leukemias. This finding became useful in the differential diagnosis of doubtful cases of pernicious anemia, and furthermore, as described elsewhere, it showed that the so-called liver treatment of pernicious anemia, while improving the morphological blood picture of patients and their general condition, did not destroy the toxic substances present in their blood. However, exposure of their blood both in vitro and clinically to ultraviolet radiation did produce a complete detoxification of pernicious anemia toxin. Another condition in which a toxic substance was demonstrated in the blood of human beings by plant physiological methods is leprosy (5). Still another disease was trachoma (6). This grave eye disease which was originally regarded as a local inflammation of the conjunctiva was thus proven to be a systemic disease with local ocular manifestations.

In the present communication the author wishes to call attention to three other conditions on which he has been working for many years and which have yielded very useful information through phytopharmacological studies, which information became of practical therapeutic and clinical

value in medical practice. The first of these is the very fatal disease known as pemphigus, a study of which was begun by the author with his late classmate and friend, the dermatologist Dr. I. R. Pels. A routine examination by phytopharmacological methods of blood sera from all kinds of dermatoses revealed that in all of them with the exception of two, the blood sera yielded a phytotoxic index the same as that of normal sera. The two exceptions were, first, leprosy which was just mentioned, and, second, the very grave disease with ninety per cent mortality known as pemphigus (7). Pemphigus is generally regarded as a skin disease but the recent studies by the present writer and his collaborators have definitely established that it is no more of a skin disease than smallpox. In other words, it is a systemic illness giving a characteristic toxic reaction of the blood and affect-

TABLE 2. *Comparison of Blood Sera.*

	Phytotoxic Index		Phytotoxic Index
	per cent		per cent
1. Normal human serum	70–75	13. Mononucleosis	80
2. Menstrual	51–60	14. Bacterial Infections	70–75
3. Pernicious Anemia	44–57	15. Eclampsia	75
4. Pemphigus	48–60	16. Syphilis	81
5. Leprosy	47	17. Tuberculosis	78
6. Trachoma	48	18. Scarlet Fever	79
7. Severe Secondary Anemia	65–69	19. Measles	80
8. Carcinoma	70	20. German Measles	80
9. Malarias	70	21. Varicilla	80
10. Banti's Disease	75	22. Post Puerperal (12 wks)	80
11. Lymphatic Leukemia	70	23. Vaccima virus	80
12. Myelagenous Leukemia	70	24. Herpes Simplex	84

ing the patient's constitution very profoundly, and is accompanied by characteristic bullae and other skin lesions. So striking is the phytopharmacological action of pemphigus serum that the so-called Macht-Pels reaction or test is now generally accepted as a valuable diagnostic criterion for this disease, and specimens for diagnosis are received in the author's laboratory almost every week. A further advance in the knowledge of this disease was made more recently by the writer in association with the late radiologist, Dr. Marcus Ostro of the Sinai Hospital, Baltimore, when they discovered that when pemphigus serum in vitro is irradiated with especially filtered hard X-rays for a few minutes the toxicity of the serum is destroyed (8). This finding *in vitro* was the starting point of a new therapeutic procedure in treating some patients afflicted with this hopeless disease, and it was found that cautious irradiation of these patients with deep X-rays in small doses over the spleen and liver produced a detoxification of their blood which was followed by more or less clinical improve-

ment in their general condition and skin lesions. In this way at least half-a-dozen patients have been saved from almost certain death by these phytopharmacological discoveries. In table 3 is given a protocol of a remarkable case of pemphigus, that of Mrs. J. B. who was in a moribund condition, and who was saved by treatment with small doses of deep X-rays, filtered through a composite filter of 2 mm. copper and 1 mm. of aluminum. Such cases are described in detail by Macht and Ostro in a medical journal.

A second important condition in which phytopharmacology has led to

TABLE 3. *Effect of Radiotherapy on a Case of Pemphigus, showing change in Phytotoxicity.*

PROTOCOL B.
Pemphigus Case Mrs. B.

Date	Phytotoxic Index	Date	Phytotoxic Index
Dec. 4, 1945 In very bad shape, moribund	50%	Mar. 4	90%
Dec. 6 Radiated ant. spleen 105r comp. filter 2 Cu		Mar. 18	72%
Dec. 7	61%	Mar. 25 Radiated liver 42r	
Dec. 8 Radiated ant. spleen 105r		Mar. 27	83%
Dec. 9	75%	April 4 Definite improvement	70%
Dec. 10 Radiated liver 110r		April 11 Definite improvement	71%
Dec. 13	78%	April 15 Discharged in greatly improved condition	72%
Dec. 19	88%	May 23 Well	69%
Dec. 27	89%	June 13 Well	68%
Discharged improved, no lesions.		July 15 Well	70%
Jan. 8, 1946 Few blebs reappearing	62%	Aug. 21 Well	80%
Feb. 13 Readmitted, relapse	50%	Sept. 23 Well	70%
Feb. 18 Radiated ant. spleen 63r		Nov. 6 Well	71%
Feb. 20 Radiated ant. spleen 63r	75%	Mar. 13, 1947	71%
Feb. 22 Radiated liver 63r		May 15 Quite well	72%
Feb. 26	90%	June 9 Quite well	72%
		Oct. 10 Quite well	72%
		Mar. 1, 1948 Quite well	72%
		Dec. 23 Entirely well	72%
		Mar. 1, 1949 Quite well	72%

interesting discoveries which promise to become of clinical value are studies on the pharmacology of the bolod sera from all kinds of mental patients which the present writer has been carrying on for the last ten years with the aid of physicians from various state hospitals for the insane. It was discovered by him that all psychoses, whether the so-called organic psychoses or the so-called functions psychoses, yield toxic reactions when examined by his special phytopharmacological methods. Thus toxic reactions were produced by blood sera from manic-depressive patients, schizophrenics, involutional melancholia, paresis, and other insanities. In contrast to these, ordinary neurological, neurasthenic, and psychoneurotic patients yielded blood sera which behave exactly like normal human serum. The importance of these findings will be stressed by the writer in a more

detailed communication to be presented in the near future before special medical societies, but it is obvious that these findings promise to become a valuable aid both in differential diagnosis of true insanities and also as a starting point of criterion for evaluating therapeutic procedures. In table 4 are given the phytotoxic readings obtained by the writer with the blood sera of some psychotic patients. Such studies, owing to their obvious importance, have been in progress for a number of years, and will be presented in detail in the near future.

In the last three years the author has been interested in another very baffling though not fatal disease, namely, psoriasis. This is a very troublesome and disagreeable skin affliction concerning which very little is known and the writer has been fruitlessly seeking for an approach to the pathology of this disease. Recently, a new approach was devised and promises

TABLE 4. *Phytotoxic Indices of Some Psychotic Blood Sera.*

Patient	Diagnosis	Phytotoxic Index per cent
E. S.	Schizophrenia, catatonic hypokinetic type	44
H. R.	Schizophrenia, catatonic hypokinetic type	42
F. N.	Schizophrenia, catatonic hyperkinetic type	58
D. H.	Schizophrenia, catatonic hyperkinetic type	58
C. H.	Involutional melancholia type	52
M. R.	Involutional melancholia type	50
B. B.	Manic-depressive type	54
J. H.	Manic-depressive type	57

to be of considerable value both in diagnosing psoriasis and as a criterion in evaluating various treatments employed by physicians for this condition. The approach was through *phytopathological* lines. All of the phytopharmacological studies on blood were hitherto carried on by him by employing normal and healthy *Lupinus albus* seedlings. Now, it is well known that various plant physiologists and especially those in Russia have reported a remarkable success in agricultural and horticultural experiments by the employment of so-called "*yarovized*" plants. This phenomenon of "yarovization" is perhaps better known among English-speaking physiologists as "*vernalization.*" A simple method of producing yarovized seedlings is by exposure of such seedlings for a period of twenty-four hours to low temperatures, but not temperatures low enough to kill the plants. By employing such vernalized seedlings the author recently demonstrated a marked difference in the phytotoxic indices given by solutions of the well-known drug ethyl-carbamate or *urethane* which is now being studied by medical investigators in connection with leukemia and cancerous growths. It was found that whereas even quite concentrated solutions of urethane

inhibit the root growth of ordinary *normal* lupine seedlings but very little indeed, a markedly phytotoxic action was exerted by very much more dilute concentrations of the same drug on vernalized seedlings. Employing the same technique in studying the blood sera of psoriasis, the author succeeded in demonstrating a definite phytotoxic effect produced by psoriasis serum on vernalized plants although normal plants were not affected by such sera at all and in fact gave higher indices even than normal blood sera. In this way already a series of psoriatic cases were studied and in

TABLE 5. *Psoriasis Sera Versus Control Sera.*

	Psoriasis Sera			Control Sera			
Normal Plants	Phytotoxic Index	Vernalized Plants	Phytotoxic Index	Normals Plants	Phytotoxic Index	Vernalized Plants	Phytotoxic Index
	per cent		per cent		per cent		per cent
No. 1	83		65	Normal human serum	75		75
2	70		49	" " "	77		70
3	82		51	" " "	71		71
4	82		43	" " "	71		75
5	88		43	" " "	72		75
6	81		50	" " "	78		78
7	91		56	Cat blood serum	84		84
8	70		50	" " "	71		74
9	81		49	Carcinoma	74		75
10	99		58	B. 875	70		73
11	91		64	B. 876	71		73
12	77		53	B. 880	70		73
13	86		53	B. 883	66		69
14	81		41	Scleroderma	87		86
15	90		49	Leukemia (lymph)	78		76
16	82		57	Schizophrenia	42		48
17	76		58	Manic depressive	33		35
18	85		63	Lupus erythematosus	65		63
19	93		52	Menstrual serum	55		52
20	86		62	Pernicious anemia	55		52
21	82		45	" "	53		53
22	70		52	Pemphigus	44		44
23	73		47	"	51		53
24	92		37	"	58		54
Av. Index	82.9		Av. Index 50.7				

each case a phytotoxic effect on yarovized plants was demonstrated. Control experiments with normal blood-sera and sera from various other diseases revealed that they do not affect significantly or at all such yarovized plants. This new psoriasis test promises to be useful in evaluating the results of different methods of therapy. For instance, the author has found in vitro that psoriatic blood serum exposed to ultraviolet rays is no longer toxic for vernalized plants and it is well known that ultraviolet irradiations are employed by dermatologists in psoriatic patients. The same was

found in vitro after exposure of psoriasis sera to small doses of filtered X-rays. Finally, a detoxification was produced in vitro of psoriatic blood serum which was mixed with small doses of *sarsaponin*, an active principle found in the plant root of *Smilax* sarsaparilla[1]. Studies as to whether patients treated with various agents such as just mentioned and with other agents will yield a detoxified psoriatic blood are now in progress. It is thus seen that the opening of a new experimental approach to studying biological phenomena, namely, with the development of the new branch of biology to which the name *phytopharmacology* has been given, discoveries have been made which are both of purely scientific interest and at the same time of considerable value in studies on the diagnosis, pathology, and therapy of different clinical conditions in men.

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[1] Furnished by the courtesy of Ernst Bischoff Co., Inc., Ivoryton, Connecticut, under the name of "Sas-Par."

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ANTIBACTERIAL ACTIVITY OF SEEDLING EXTRACTS OF CULTIVATED PLANTS

HUBERT A. HARRIS

Since the first extensive survey of higher plants for antibacterial substances by Osborn (1943), similar surveys have been conducted by Huddleson, DuFrain, Barrons, and Geifel (1944), Lucas and Lewis (1944), Sanders, Weatherwax, and McClung (1945), Carlson, Bissell, and Mueller (1946), Hayes (1947), and Carlson, Douglas, and Robertson (1948).

Among these surveys are included some cultivated plants, but chiefly they involved tests on field collections of wild plants. Cultivated plants, containing antibacterial substances of therapeutic value, would, in general, have greater economic production advantages over wild plants possessing such substances because of their greater adaptability to commercial propagation.

Screening tests of higher plants for antibacterial substances have presented investigators with several difficulties. Aside from the task of collecting widely scattered plants in the field and maintaining them in a fresh condition for testing, the antibacterial substances are not always of general occurrence in the plant. Instead, it has been reported that they may be concentrated only in a single part such as the root, stem, bark, leaf, flower, fruit, or seed. Tests are therefore necessitated on each separate plant part and certain parts frequently are unavailable from field specimens. Antibacterial activity, too, has been determined to be influenced by varietal character, age of the plant, and the temperature under which the plant is growing when collected. More recently, and during the course of the present investigation, Carlson and Douglas (1948) have shown that screening tests of plants for antibacterial substances require the use of more than one solvent for extractives before a plant can be discarded as lacking antibacterial activity.

Irving, Fontaine, and Doolittle (1945) reported that lycopersicin, later renamed tomatin by them (1946), was absent in the seed of the tomato plant, but appeared in seedlings germinated in the dark and in the plant within eight days after planting. Screening tests could be partially simplified if seedlings contain antibacterial substances that persist in the mature plant. It is recognized that such substances in the mature plant may not be present in the seedling stage, but, conversely, antibacterial substances may possibly be transitory in seedlings and not occur in mature plants. This presents no serious objection, however, since one of the

chief objectives of screening tests is to discover indications of antibacterial activity for further investigation. Screening tests do not purport altogether to exhaust the antibacterial possibilities of extracts of individual plants; hence positive antibacterial activity in seedling extracts might afford valuable data.

TABLE I. *Antibacterial Activity of Seedling Extracts.*

Seedling	E. col.	S. mar.	S. lut.	S. aur.
Compositae				
Carthamus tinctorius (saffron) 661	−	++	−	−
Cynara cardunculus (cardoon) 661	−	+++P	++++	+
Cynara scolymus (artichoke) green globe 3	−	++P	+++	+
Convolvulaceae				
Ipomea noctiflora (moon flower) 376	−	−	++	−
Cruciferae				
Brassica oleracea var. *italica* (sprouting broccoli) Italian green sprouting 159	−	−	+	−
Cheiranthus cheiri (wallflower) 382	++P	+++P	+	−
Iberis umbellata (candytuft) 208	−	+++P	+++	−
**Iberis umbellata* (candytuft) 208	−	+P	++	−
Malcomia maritima (Virginian stock) 381	++P	++P	++++	+
**Malcomia maritima* (Virginian stock) 381	++P	+++P	++	+
Gramineae				
Zea mays var. *saccharata* (sweet corn) golden bantam extra early 851	−	−	++	−
Liliaceae				
Allium cepa (onion) yellow globe Danvers 77	−	−	−	+
Allium porrum (leek) large American flag 171	−	−	++	−
Rosaceae				
Geum chiloense (Mrs. Bradshaw geum) 344	+	+	−	−
Solanaceae				
Capsicum frutescens var. *grossum* (bell pepper) ruby king 97	−	−	+	−
Lycopersicum esculentum (tomato) ponderosa 133	−	−	+	−
Tropaeolaceae				
Tropaeolum peregrinum (canary bird vine) 302	−	++P	++P	−

* Antibacterial activity of mature plant extract.
−=no inhibition.
+=13–16 mm. zone of inhibition.
++=17–21 mm. zone of inhibition.
+++=22–26 mm. zone of inhibition.
++++=27–30 mm. zone of inhibition.
P=partial inhibition.

The results of a preliminary test indicated sufficient correlation between comparative tests on the occurrence of antibacterial substances in the extracts of 15 different species of seedlings and the extracts of the mature stages of these same plants to warrant a more extensive survey for antibacterial substances in cultivated plants using seedling extracts.

In this preliminary test, seedling extracts of two species of plants belonging to the Cruciferae, *Iberis umbellata* (candytuft) and *Malcomia maritima* (Virginian stock), showed comparable antibacterial activity for both the seedling extracts and the mature plant extracts as shown in table 1. The extracts of both seedlings and mature plants of 13 different species of plants showed negative antibacterial activity with all four bacterial test organisms. These plants were as follows: Convolvulaceae, *Quamoclit pinnata* (cypress vine), *Q. sloteri* (cardinal climber); Caryophyllaceae, *Dianthus chinensis* var. *heddewigii* (pinks); Labiatae, *Satureia hortensis* (summer savory); Leguminosae, *Pisum sativum* (garden pea); Linaceae, *Linum grandiflorum* var. *rubrum* (scarlet flax); Malvaceae, *Althaea rosea* (hollyhock); Nyctaginaceae, *Mirabilis jalapa* (four o'clock); Papavaraceae, *Escholtzia californica* (california poppy); Scrophulariaceae, *Delphinium hybridum* (larkspur); Solanaceae, *Petunia hybrida* (common garden petunia), *Nicotiana alata* var. *grandiflora* (flowering tobacco); Tropaeolaceae, *Tropaeolum majus* (garden nasturtium).

The present investigation reports in vitro tests for water-soluble antibacterial substances in 145 different species and varieties of cultivated plant seedlings belonging to 37 different families, using two Gram-negative and two Gram-positive bacteria as test organisms.

Materials and Methods. The various seeds[1] were planted in moist sand in greenhouse flats and the seedlings, except in a few instances of slow germination, were two to three weeks old when tested for antibacterial properties. Greenhouse temperatures were 20–30° C. The seedlings were freed of sand by several rinsings in distilled water and the entire seedlings were crushed thoroughly with mortar and pestle to extract the juice, which was tested immediately against each of the following four bacteria: *Escherichia coli:* ATCC 9739. *Serratia marcescens:* ATCC 4261. *Staphylococcus aureus: Oxford strain* H, ATCC 9144. *Sarcina lutea:* stock laboratory culture, source unknown.

The filter paper disc method of Vincent and Vincent (1944) was used to determine comparative qualitative tests of antibacterial substances in the seedling extracts. Filter paper discs (Schleicher and Schuell, no. 740-E, 12.7 mm. diam.) were saturated in the test extract, the excess fluid gently shaken off, and placed on seeded nutrient agar plates. Six discs, comprising duplicate tests of extracts from three different kinds of seedlings, were spaced equidistant on each agar plate.

Before being used for seeding the agar plates, each bacterial test organism was transferred three times on successive days to 10 ml. of nutrient broth. All broth cultures, save those of *S. marcescens,* were incubated at

[1] The seeds used in these experiments were supplied by the courtesy of the Northrup and King Seed Company, Minneapolis, Minnesota.

37° C for 16–20 hrs. The latter cultures were incubated at room temperature (23–27° C).

Nutrient agar was dispensed (20 ml.) into sterile petri dishes with a sterile pipette; the surface of the solidified agar was flooded with a culture suspension of the test organism and the excess suspension pipetted off. The surfaces of the flooded agar plates, with the covers removed, were dried for 45 min. in a 37° C incubator. The test plates were incubated at the same temperatures and for the same time intervals as for the test bacteria in nutrient broth.

Measurements were recorded to the nearest 0.5 mm. of the outside diameters of the inhibitory zones of the test organisms by means of pointed dividers under a magnifying lens.

Experimental Results. Many of the varieties of cultivated plants used in this investigation are either unknown or doubtful even to the seedsmen. Consequently, the varieties tested are listed only for certain ones, but all plants tested are more specifically referred to by the seed company's packet number which is listed after each plant name. The nomenclature employed, with few exceptions, is that of Bailey (1927).

As shown in table 1, positive tests for antibacterial activity, against one or more test organisms, were obtained with seedling extracts of 17 different species of cultivated plants belonging to nine different families.

The antibacterial substances, in general, were more effective against the Gram-positive than Gram-negative bacteria. With few exceptions, the latter organisms were only partially inhibited or not at all. The seedling extract of only one plant species, *Malcomia maritima* (Virginian stock), showed some activity against all four test organisms.

Seedling extracts of the following plant species or varieties exhibited negative antibacterial activity with all four test organisms.

Amaranthaceae
- *Celosia argentea* (plumed cockscomb) 340
- *Gomphrena globosa* (globe amaranth) 398

Apocynaceae
- *Vinca rosea* (Madagascar periwinkle) 380

Balsaminaceae
- *Impatiens balsamina* (garden balsam) 202

Boraginaceae
- *Anchusa capensis* (bluebird) 226
- *Borago officinalis* (borage) 3379
- *Cynoglossum amabile* (cynoglossum) 212
- *Myosotis sylvatica* (forget-me-not) 364

Campanulaceae
- *Campanula medium* var. *calycanthema* (cup and saucer Canterbury bell) 338
- *Lobelia erinus* var. *compacta* (lobelia) 345

Capparidaceae
- *Dianthus barbatus* (sweet William) 4851
- *Dianthus caryophyllus* (carnation) 333

Dianthus chinensis var. *heddewigii* (pinks) 216
Gypsophila elegans (baby's breath, crimson) 264
Gypsophila elegans (baby's breath, white) 214
Chenopodiaceae
Beta vulgaris (beet) Detroit dark red turnip 3
Beta vulgaris var. *cicla* (Swiss chard) Fordhook giant 8
Beta vulgaris var. *crassa* (sugar beet) U. S. strain 199
Kochia trichophylla (summer cypress) 215
Spinacia oleracea (spinach) Bloomsdale 118
Compositae
Ageratum houstonianum (floss flower) 359
Artemesia absinthium (wormwood) 661
Bellis perennis (English daisy) 358
Brachycome iberidifolia (Swan river daisy) 337
Calendula officinalis (pot marigold) 204
Callistephus chinensis (China aster) 301
Centaurea cyanus (bachelor's button) 203
Chrysanthemum maximum (Shasta daisy) 353
Cichorium endiva (endive) green curled 166
Cichorium intybus (chicory) witloof 163
Coreopsis coronata (tickseed) 276
Coreopsis lanceolata (coreopsis) 271
Cosmos bipinnatus (cosmos) 210
Dahlia pinnata (dahlia) 387
Gaillardia aristata (gaillardia) 375
Helianthus annuus (sunflower) 232
Lactuca sativa (lettuce) black-seeded Simpson 46
Lactuca sativa var. *romana* (cos lettuce) Paris white cos 184
Tagetes erecta (African marigold) 346
Taraxacum officinale (common dandelion) improved thick leaf 177
Thelesperma hybridum (cosmidium) 257
Tragopogon porrifolius (salsify) Sandwich Island 115
Zinnia grandiflora (zinnia) 289
Convolvulaceae
Ipomoea noctiflora (moonflower) 376
Quamoclit pinnata (cypress vine) 305
Quamoclit sloteri (carbinal climber) 339
Cruciferae
Alyssum maritimum (sweet alyssum) 223
Brassica caulo-rapa (kohl-rabi) early white Vienna 169
Brassica juncea (Chinese mustard) Chinese broad leaf 173
Brassica napobrassica (rutabaga) American purple top 151
Brassica oleracea var. *acephala* (collards) Georgia 164
Brassica oleracea var. *acephala* (kale) dwarf green curled 168
Brassica oleracea var. *botrytis* (cauliflower) snowball No. 16 domestic 30
Brassica oleracea var. *capitata* (cabbage) late flat Dutch 16
Brassica oleracea var. *gemmifera* (Brussel's sprouts) Long Island improved 179
Brassica pekinensis (Chinese cabbage) Chihili 195
Brassica rapa (turnip) purple top strap leaved 145
Eruca sativa (roquette) 661
Lepidium sativum (garden cress) curled 86
Raphanus sativus (radish) early scarlet turnip 106
Cucurbitaceae
Citrullus vulgaris (watermelon) Kleckley's sweets 64
Cucumis melo var. *reticulatus* (muskmelon) Rocky Ford 55
Cuburbita pepo (field pumpkin) early sweet sugar 101

Cucurbita pepo var. *condensa* (summer squash) giant summer crook neck 121
Cucurbita pepo var. *ovifera* (ornamental gourd) 365
Cucumis sativus (cucumber) Boston pickling 37

Dipsaceae
Scabiosa atropurpurea (mourning bride) 236

Euphorbiaceae
Euphorbia marginata (snow on the mountain) 270
Ricinus communis (castor bean) 250

Hydrophyllaceae
Nemophila menziesii var. *insignis* (baby blue eyes) 277

Labiatae
Hyssopus officinalis (hyssop) 661
Melissa officinalis (lemon balm) 199
Nepeta cataria (catnip) 123
Ocimum basilicum (basil) 51
Origanum majorana (sweet marjoram) 156
Rosmarinus officinalis (rosemary) 3376
Salvia officinalis (sage) 157
Satureia hortensis (summer savory) 193
Thymus vulgaris (thyme) 158

Leguminosae
Dolichos lablab (hyacinth bean) 265
Lathyrus odoratus (sweet pea) 329
Lupinus hartwegii (lupine) 266
Phaseolus vulgaris var. *humilis* (bush bean) giant stringless green pod 809
Pisum sativum (garden pea) little gem 835

Liliaceae
Asparagus officinalis (garden asparagus) Mary Washington 50

Linaceae
Linum grandiflorum var. *rubrum* (scarlet flax) 274

Malvaceae
Althaea rosea (hollyhock) 307
Hibiscus esculentus (okra) mammoth long green podded 174
Hibiscus sp. (hibiscus) 219

Nyctaginaceae
Mirabilis jalapa (four o'clock) 213

Onagraceae
Clarkia elegans (clarkia) 363
Godetia grandiflora (godetia) 272

Papavaraceae
Eschscholtzia californica (California poppy) 205
Papaver rhoeas (Shirley poppy) 234

Polemoniaceae
Phlox drummondii (phlox) 378

Portulaceae
Portulaca grandiflora (rose moss) 348

Polygonaceae
Rheum rhaponticum (garden rhubarb) mammoth Victoria 176
Rumex acetosa (sorrell) 3374

Ranunculaceae
Aquilegia longissima (long spurred columbine) 341
Delphinium hybridum (larkspur) 217
Nigella damascena (love in the mist) 273

Resedaceae
Reseda odorata (common mignonette) 222

Rutaceae
Ruta graveolens (rue) 661
Scrophulariaceae
Antirrhinum majus (common snapdragon) 354
Digitalis purpurea (foxglove) 343
Linaria maroccana (linaria) 389
Nemesia strumosa (nemesia) 350
Solanaceae
Nicotiana alata var. *grandiflora* (flowering tobacco) 377
Petunia hybrida (common garden petunia) 243
Salpiglossis sinuata (painted tongue) 319
Schizanthus wisetonensis (butterfly bush) 237
Solanum melongena var. *esculentum* (eggplant) black beauty 165
Tropaeolaceae
Tropaeolum majus (garden nasturtium) 225
Umbelliferae
Anethum graveolens (dill) 155
Apium graveolens (celery) golden self-blanching 31
Apium graveolens var. *rapaceum* (celeriac) giant Prague 35
Carum carvi (caraway) 661
Coriandrum sativum (coriander) 2101
Daucus carota (carrot) Chantenay 28
Ferula communis (giant fennel) finocchio 2507
Foeniculum dulce (sweet fennel) 661
Pastinaca sativa (parsnip) improved hollow crown 92
Petroselinum hortense (parsley) dark moss curled 88
Pimpinella anisum (anise) 3380
Trachymene caerulea (blue lace flower) 336
Verbenaceae
Verbena hybrida (common garden verbena) 249
Violaceae
Viola cornuta (bedding pansy) 311

Correlation of Results With Other Investigations. Seven of the 15 plant species whose seedling extracts showed antibacterial action in the present investigation have been tested for antibacterial activity by other investigators. These seven are: *Brassica oleracea* var. *italica* (sprouting broccoli), *Cheiranthus cheiri* (wallflower), *Malcomia maritima* (Virginian stock), *Zea mays* var. *saccharata* (sweet corn), *Allium cepa* (onion), *Capsicum frutescens* var. *grossum* (bell pepper), and *Lycopersicum esculentum* (tomato).

Osborn (1943) found that the extracts of the seeds of white and purple varieties of sprouting broccoli inhibited *E. coli* and *S. aureus.*

She reported also that the vegetative extracts of the wallflower inhibited these same organisms as a result of enzyme action. Sanders (1946) reported that cheiroline, a substance extracted from the seeds of the wallflower, yielded negative effects on certain pathogenic fungi.

The extracts of the seeds of Virginian stock were reported by Osborn (1943) to inhibit *E. coli* and *S. aureus.*

The juice of sweet corn was reported by Little and Grubaugh (1946) to be inhibitive to *Eberthella typhosa, E. coli, Salmonella paratyphi* A,

S. aureus, Erwinia carotovora, Phytomonas stewartii, and two forms of the fungus *Fusarium oxysporum,* f. *melonis* and f. *niveum.*

The antibacterial properties of the onion, especially onion vapors, have been well established by Brown (1917), Sarti (1919), Walker, Lindegren, and Bachman (1925), Lovell (1937), Vollroth, Walton, and Lindegren (1937), Ingersoll, Vollroth, Scott, and Lindegren (1938), Fuller and Higgins (1940), Tokin (1943), Huddleson, DuFrain, Barrons, and Giefel (1944), Kovalenok (1944), Pederson and Fisher (1944), Toroptsev and Filatova (1944), Carpenter (1945), and Sanders, Weatherwax, and McClung (1945).

Seedling extracts of pepper were reported by Tokin (1943) to exhibit powerful bactericidal properties and to affect protozoa after prolonged exposure.

Irving, Fontaine, and Doolittle (1945) reported the extraction of an antibacterial substance, later termed tomatin (1946), from the tomato plant. This substance has become the subject of further investigations in a series of papers: Fontaine, Irving, and Doolittle (1947), Irving (1947), Fontaine, Ma, Poole, Porter, and Naghski (1947), Ma and Fontaine (1948), and Fontaine, Irving, Ma, Poole, and Doolittle (1948). Extracts of tomato plants were found by Little and Grubaugh (1946) to inhibit *S. paratyphi* A, *S. aureus,* and three forms of *Fusarium oxysporum:* f. *conglutinans,* f. *lycopersici,* and f. *melonis.* Pederson and Fisher (1944) and Carpenter (1945) reported little, if any, inhibition by tomato juice of certain bacteria.

Several of the species of plants whose seedling extracts yielded antibacterial activity in the present investigation have been tested for antibacterial properties by a number of other investigators.

Sherman and Hodge (1936) found juices of cabbage and turnip to inhibit *Aerobacter aerogenes, E. coli, Phytomonas campestris,* and natural mixed bacterial flora from the exterior of plant tissues. Negative antibacterial activity resulted with the juices of carrot, cucumber, and parsnip on the same bacteria.

Osborn (1943) found that the extracts of the seeds of Brussels sprouts, borecole, broccoli, cabbage, cauliflower, and kohl-rabi inhibited *E. coli* and *S. aureus,* but extracts of the vegetative parts of these same plants produced only a slight partial inhibition of *S. aureus.*

Huddleson, DuFrain, Barrons, and Geifel (1944) reported that the juice of rhubarb inhibited *Brucella abortus* and *S. aureus.* The active substance was present only in the petiole of the plant.

Lucas and Lewis (1944) were unable to demonstrate antibacterial action of cabbage and turnip extracts against *E. coli, Phytomonas campestris, P. phaseoli,* and *S. aureus.*

Pederson and Fisher (1944) demonstrated the antibacterial activity

of cabbage juice toward certain Gram-negative bacteria occurring on the surface of cabbage leaves, *E. coli*, and *S. aureus*. Little, if any, inhibition was noted with juices of broccoli, Chinese cabbage, carrot, cauliflower, celery, cucumber, and turnip on the same bacteria.

Carpenter (1945) obtained negative antibacterial results with the juices of beet and celery on *E. coli*, *Pseudomonas aeruginosa*, and *S. aureus*.

Sanders, Weatherwax, and McClung (1945) found the extract of chicory to inhibit *E. coli* and *S. aureus*. The extracts of asparagus and the wild carrot failed to inhibit either organism.

Little and Grubaugh (1946) obtained inhibition of *E. typhosa*, and *S. paratyphi* A with bean juice. The juice of cauliflower inhibited *E. typhosa* and cucumber juice inhibited *Erwinia carotovora*.

Hayes (1947) found little or no inhibition of *E. carotovora*, *E. coli*, *Phytomonas tumefaciens*, and *S. aureus* with the extracts of asparagus, carrot, chicory, and dandelion.

Carlson, Douglas, and Robertson (1948) obtained negative antibacterial activity using aqueous extracts of mature sunflower plants against *E. coli* and *S. aureus*. However, ether extracts of the flowers completely inhibited *S. aureus.*, but this organism was only partially inhibited by similar extracts prepared from the root-stem and leaf.

Discussion. The present investigation shows that seedling extracts are of value for antibacterial screening tests of higher plants and that such seedling extracts can be tested quite readily. The use of several extractive solvents, as recommended by Carlson and Douglas (1948), would be applicable with seedling extracts if the filter paper disc method of testing is employed since this method requires the use of very small quantities of test extracts.

The occurrence of antibacterial substances in seedling extracts compares favorably on a percentage basis with the screening tests of some other investigators employing the use of extracts from mature plants or plant parts. The results of some of these latter surveys show approximately the following percentages of plants possessing antibacterial properties to the total number of plants tested: Atkinson and Rainsford (1946), 15 per cent; Hayes (1947), 16 per cent; Sanders, Weatherwax, and McClung (1945), 10 per cent; Carlson, Bissell, and Mueller (1946), 4 per cent; and Carlson, Douglas, and Robertson (1948), 20 per cent. The results of the present investigation show 12 per cent.

The in vitro tests of the seedling extracts of cultivated plants indicate the presence of a few hitherto unreported antibacterial substances apparently worthy of further investigation.

SUMMARY

1. Data are presented for *in vitro* screening tests for antibacterial substances in the seedling extracts of cultivated plants belonging to 37 different families using *Escherichia coli, Serratia marcescens, Sarcina lutea,* and *Staphylococcus aureus* for test organisms.

2. The use of seedling extracts is of value in screening tests of higher plants for antibacterial substances.

3. Approximately 12 per cent of the total number of different kinds of plant seedling extracts tested evidenced antibacterial properties.

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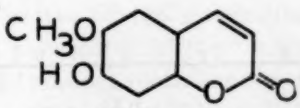
THE ISOLATION OF SCOPOLETIN, A BLUE-FLUORESCING COMPOUND FROM OAT ROOTS

RICHARD H. GOODWIN AND FREDERICK KAVANAGH

In an investigation of the fluorescence of roots (Goodwin & Kavanagh 1948), it was found that oats were a very rich source of a blue-fluorescing material, which was readily extracted with n-butanol, acetone, and other solvents. The extracts were separated into a number of different fractions, each of which exhibited different chemical properties. The fractions were distinguished by the relation between fluorescence and hydrogen ion concentration of the solution. The shapes of the pH-fluorescence curves were very distinctive.

The pH-fluorescence curves of a number of naturally-occurring organic compounds have been determined (Kavanagh & Goodwin 1949; and unpublished). Attempts to match these curves with those of the fluorescing fractions from oat roots proved unsuccessful until we recently obtained a sample of scopoletin (6-methoxy-7-hydroxy coumarin or 6-methoxy-7-hydroxy 1:2 benzopyrone).



The pH-fluorescence curve of this compound had a shape very similar to that of one of our fractions (fraction *b*) from oat roots. A substance having some properties identical to those of scopoletin was then prepared from oat roots by a procedure recommended for the isolation of scopoletin.

Method of Extraction. The roots, amounting to 120 grams, were harvested from 3,300 five-day-old oat seedlings, *Avena sativa* var. Victory, germinated on filter paper in the laboratory. These were extracted by the method described by Best (1944). The roots were covered with 240 ml. of .05 N H_2SO_4 and 100 ml. of chloroform and allowed to stand overnight. The chloroform was decanted and another 100 ml. of chloroform were added. After another twelve hours the second batch of chloroform was removed. The two chloroform fractions were combined and shaken repeatedly with 25 ml. portions of .005 M ammonium hydroxide. The ammonium hydroxide fractions were combined, acidified to about pH 2.5, and extracted with five portions of 50 ml. of chloroform. The chloroform fractions were then combined, dried over neutral calcium chloride, and filtered, and a small sample was removed for fluorometric study. The remainder was evaporated to dryness under vacuum. Approximately 3.8 mg.

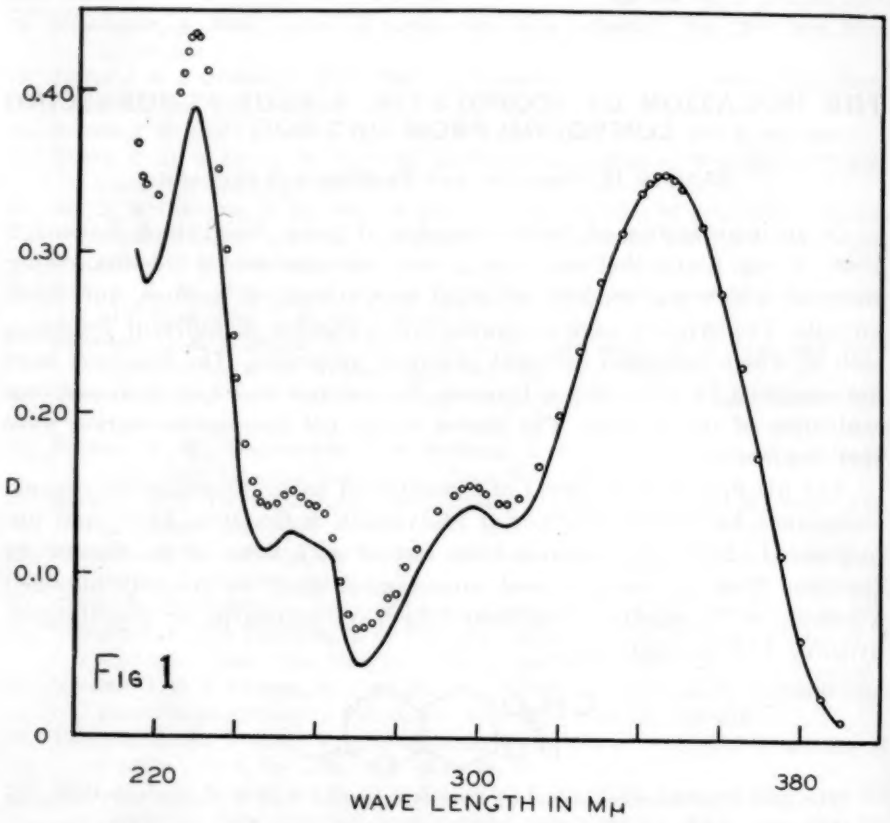

0.40
0.30
0.20
D
0.10
0
220
300
380
WAVE LENGTH IN Mμ

FIG 1

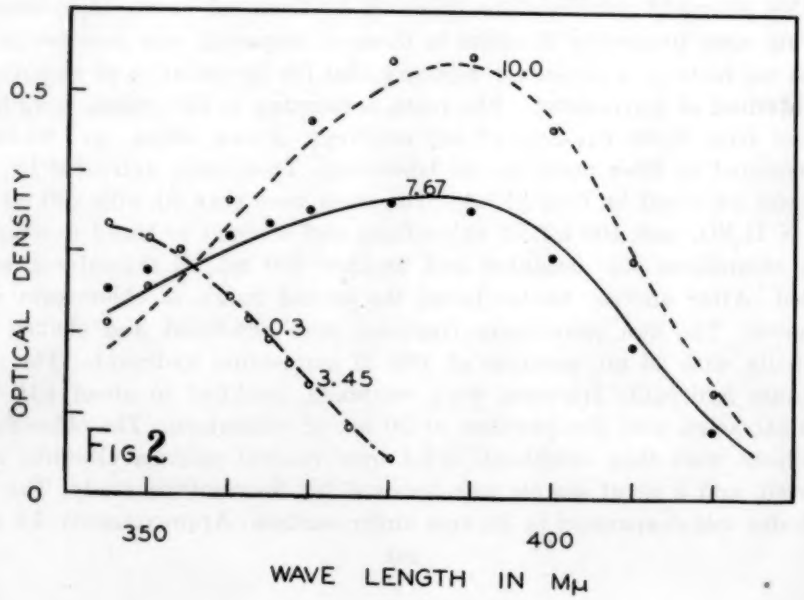

0.5
OPTICAL DENSITY
0
10.0
7.67
0.3
3.45
350
400
WAVE LENGTH IN Mμ

FIG 2

of material were obtained. The residue was dissolved in 5 ml. benzene and put on a column of alumina 80 to 200 MM (alumina adsorption, Fisher Scientific Company). The brightly blue fluorescent material was strongly adsorbed at the top of the column and was washed repeatedly with benzene. The column was then washed with ethyl ether and subsequently with chloroform, but the fluorescent material remained at the top of the column during these washings. The alumina on which the fluorescent material was adsorbed was mechanically removed and eluted with .005 M ammonium hydroxide. The ammonium hydroxide was acidified to pH 3 and extracted with chloroform. The chloroform was dried over neutral calcium chloride, filtered, and the chloroform again evaporated under vacuum. The residue recovered by this procedure weighed 1.4 mg.

Physical Properties. *Absorption Spectra.* The absorption in the ultra-violet of the substance isolated from oat roots, as described above, was then compared with that of scopoletin. Our scopoletin sample, generously supplied by Dr. W. A. Andreae of the University of New Brunswick, was originally isolated from the roots of *Gelsemium sempervirens* and came from Dr. Léo Marion, National Research Council, Canada. The absorption curve of scopoletin (fig. 1) was measured in the spectral range between 210 mμ and 400 mμ at a concentration of 2.5×10^{-5} molar in 95 per cent ethanol, using a Beckman spectrophotometer. The measurements were made with the narrowest slit widths of the spectrophotometer and correction was made for the optical asymmetry of the "matched" quartz cells.

The substance isolated from oat roots was also prepared in ethanol at a concentration of 7.5 micrograms per milliliter. It was immediately obvious that the absorption curve was nearly identical with that of scopoletin. The assumption was then made that the impurities in the root extract did not absorb light at 347 mμ and the two curves were made to coincide at that wave length. When this was done, the curves differed by less than two per cent in absorption at wave lengths between 325 and 400 mμ. The difference between the curves increased steadily with decreasing wave-length. The extra absorption could be caused by a mixture of contaminating substances and shows only "end-absorption"—no peaks. If scopoletin is the substance in the root extract responsible for the 347 mμ peak, then 52 per

Explanation of figures 1 and 2

FIG. 1. Absorption spectra (optical density as ordinate and wave length in millimicra as abscissa) of an alcoholic solution of scopoletin at a concentration of 2.5×10^{-5} molar (continuous curve) and of the root substance (circles). The two curves were made to coincide at 347 mμ. FIG. 2. Absorption spectra of scopoletin at a concentration of 2.5×10^{-5} molar (continuous curve) and of the root substance (circles) when measured in buffers with a pH of 0.3, 3.45, 7.67, and 10.0. The concentration of the root substance was such that the absorption at 347 mμ when measured in alcohol was the same as that of 2.5×10^{-5} molar scopoletin.

cent of the weight is scopoletin, and the other 48 per cent of the solids accounts for one-eighth or less of the total absorption at any wave length.

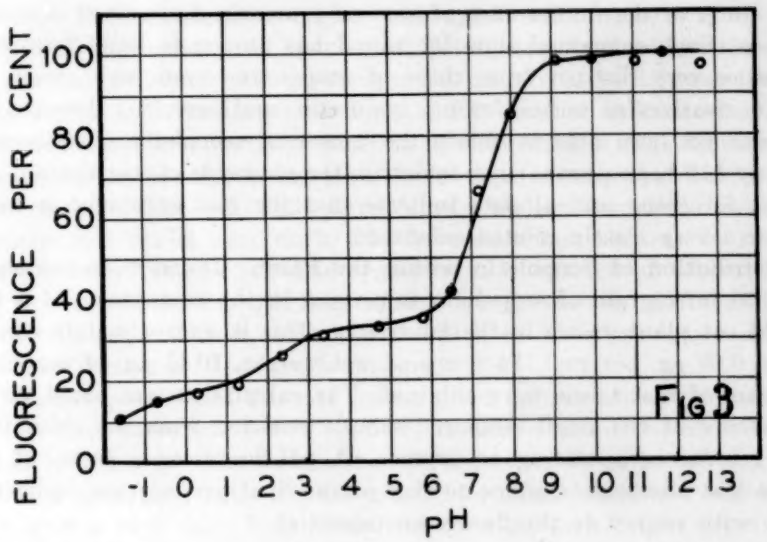
Qualitatively the two curves are strikingly similar with the peaks, valleys and plateaus occurring at identical wave lengths. The quantitative measurements on scopoletin are in good agreement with those of Andreae (1948).

The absorption spectra of scopoletin and the root extract in the region between 345 and 425 mμ (fig. 2) were measured in 1 N HCl (pH 0.3), in 0.1 M phosphate at pH 3.5 and pH 7.5, and in 0.5 per cent sodium bicarbonate-carbonate (pH 10.0). The scopoletin was measured at a concentration of 2.5×10^{-5} molar. The root substance was measured at the concentration that gave the same absorption in alcohol at 347 mμ as 2.5×10^{-5} molar scopoletin. The curves of both substances obtained at pH 0.3 and pH 3.5 were identical. The agreement in the other buffers is not as good, with deviations as large as 10 per cent. In general, the agreement is sufficiently exact to indicate that the absorption of the two solutions is due to the presence of the same or very closely related substances.

Fluorescence. The relationship between fluorescence and hydrogen ion concentration for scopoletin and for the root extract was determined by the method previously reported (Kavanagh & Goodwin 1949).[1] Sulfuric acid solutions of known normality (Michaelis & Granick 1942) were used to obtain pH values lower than 2. With a Corning 5970 lamp filter and a Corning 3060 + 3389 photocell filter, scopoletin at a concentration of 0.1 μg./ml. and the root extract at a concentration of 0.192 μg./ml. (a concentration which would show an absorption equal to that of 0.1 μg. /ml. scopoletin at 347 mμ) gave pH-fluorescence curves which were essentially identical. The potentiometer readings for the two substances were within the limits of experimental error at each pH. With the fluorimeter set to 100 with a 1 μg./ml. quinine sulfate standard, the scopoletin solution had a fluorescence of 189 at pH 9.35. The pH-fluorescence curves (fig. 3) obtained with a Corning 5860 + 738 lamp filter and a Corning 3060 photocell filter were also identical. Scopoletin at a concentration of 0.1 μg./ml. gave a reading of 148 under these conditions.

The identity of the two sets of pH-fluorescence curves indicates either that scopoletin and the root substance are identical or that the latter has exactly the same fluorescent efficiency as scopoletin and is quenched to the same extent by oxygen and by the buffer salts. Furthermore, the two substances show the same bright blue fluorescence throughout the pH range, and both decompose at the same rate in two buffers (pH 11.0 and 8.0), when irradiated by the 366 mμ lines of the mercury lamp.

[1] The Klett fluorimeter-colorimeter used in this investigation was purchased with a grant to the senior author from the Rumford Committee of the American Academy of Arts and Sciences.



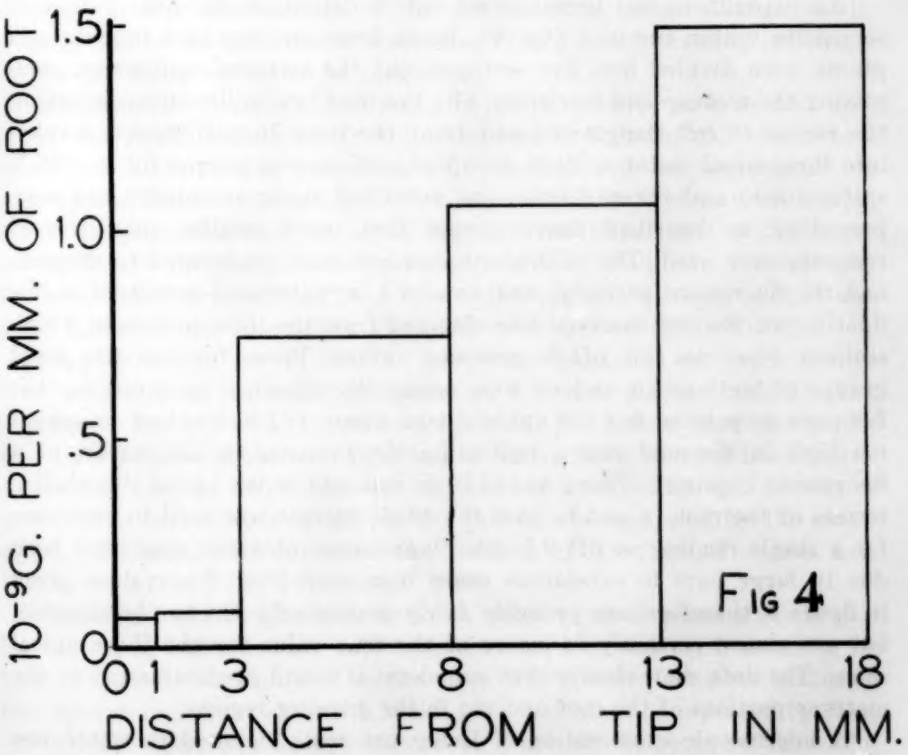


FIG. 3. The relationship between relative fluorescence and pH for scopoletin (continuous line) and for the root substance (circles). Lamp filter, Corning 5860+738; photocell filter, Corning 3060. FIG. 4. The distribution of scopoletin within the roots of 4-day-old *Avena* seedlings.

A study of the fluorescence of various coumarin derivatives is now in progress. Each compound thus far tested has proved to have fluorescent properties very distinct from those of scopoletin—even such closely related derivatives as umbelliferone, aesculetin, and aesculin. However, we have not yet been able to obtain the isomer of scopoletin (6-hydroxy-7-methoxy 1:2 benzopyrone) nor fabiatrin, the glucoside of scopoletin.

The foregoing optical data indicate that the root substance is scopoletin or a very closely related substance.

Distribution of Scopoletin within the Plant. It has been calculated that 0.38 microgram of scopoletin is present in the root-system of a five-day-old oat plant grown in the laboratory. This is approximately equivalent to 0.09 μg. per root. In terms of wet weight, 10.43 μg. of scopoletin per gram of root tissue were obtained. The calculation was based on the fluorescence of the small measured sample removed from the chloroform extract before evaporating to dryness. A pH-fluorescence curve of this sample was identical in shape to that of the final preparation, indicating purity with respect to the fluorescent material.

An experiment has been carried out to determine the distribution of scopoletin within the root (fig. 4). Roots from one hundred four-day-old plants were divided into five sections: (a) the terminal millimeter, comprising the rootcap and meristem; (b) the next two millimeters, including the region of cell elongation; and (c-e) the next 15 millimeters, divided into three equal sections. Each group of sections was ground up in 0.05 N sulfuric acid and the scopoletin was extracted, using essentially the same procedure as described above, except that much smaller quantities of reagents were used. The chloroform extracts were evaporated to dryness, and the fluorescent material was dissolved in water and measured in the fluorimeter. Enough material was obtained from the three groups of 5-mm. sections (c-e) to run pH-fluorescence curves. Those for the two basal groups of sections (d and e) were essentially identical in shape to that for pure scopoletin, but the apical 5-mm. group (c) had values somewhat too high in the acid range, indicating the presence in the extract of a fluorescing impurity. There was so little material in the apical three millimeters of the root (a and b) that the whole extract was used in each case for a single reading at pH 9.1. The fluorescence obtained may have been due in large part to substances other than scopoletin. The values given in figure 4, therefore, are probably fairly accurate for the two basal zones, but are almost certainly in excess of the true value for the three apical zones. The data show clearly that scopoletin is found predominantly in the maturer portions of the root and not in the growing regions.

A microscopic examination of living oat roots mounted in water was made using a dark-field condenser and the 366 mμ line from a mercury

arc lamp. The meristematic region (the apical millimeter) was so brilliantly blue-fluorescent that histological detail was obscured. In the root-cap cells and epidermal cells adjacent to the meristem, the fluorescence was chiefly confined to the larger vacuoles and to smaller cellular structures which may have been vacuoles, plastids or mitochondria. The fluorescence of these smaller structures was often much more intense than that of the larger vacuoles. The cell walls were faintly bluish-fluorescent, while the cytoplasm and nuclei were dark. The fluorescent material can be readily extracted from the meristematic region with a number of solvents and gives a very distinctive pH-fluorescence curve, entirely different from that of scopoletin. This is the material which we have previously characterized as fraction *a* (Goodwin & Kavanagh 1948).

The older portions of the root show a much fainter bluish fluorescence, but if sodium carbonate buffer (pH 11) is drawn under the cover slip, a marked increase in this fluorescence is observed. Vacuoles of the root hairs and epidermal cells become a brighter blue, while the cell walls, which were a very faint blue in neutral solution, turn bright green. The increase in blue fluorescence of the cell contents under alkaline conditions is due to the presence of scopoletin. The green-fluorescing substance in the cell walls has yet to be identified.

Discussion. Coumarin and many of its derivatives such as umbelliferone, aesculetin, scopoletin, fraxetin, daphnetin, limettin and other more complicated compounds are frequently encountered as constituents of plants (see Sethna & Shah 1945). A number of the hydroxy coumarins occur as glucosides and, as such, may become important in the sugar metabolism of the plant. Aesculin, the glucoside of aesculetin, for example, has been found to constitute from four to five per cent of the weight of air-dried leaves of the Australian plant *Bursaria spinosa* (Dick 1943).

Scopoletin and its glucoside, fabiatrin (scopolin, murrayin), have been isolated from a number of species of flowering plants (table 1). The Solanaceae are particularly well represented. We believe that this is the first time scopoletin has been reported as having been isolated from monocotyledons, and we suspect that it may have a much wider distribution in plants than is indicated in table 1. Its presence is suggested by the shape of the pH-fluorescence curves of raw extracts of *Hordeum*, *Triticum*, and *Pisum* roots (Goodwin & Kavanagh 1948).

Scopoletin and its glucoside occur in many types of plant structures—leaves, stems, bark, flowers, tubers, and roots; but scopoletin seems to be most frequently accumulated in the roots. The histological distribution of blue-fluorescing material in the healthy tobacco plant is reported by Best (1948). He finds this fluorescence to be markedly brighter in the endodermis of the root-stock, stem, and leaf veins than in the other tissues of

these organs, while the small roots are fluorescent throughout. The blue fluorescence which he reports may not all be due to the presence of scopoletin, however.

In tobacco and potatoes scopoletin is produced in increased amounts as a consequence of certain virus infections. In tobacco infected with tomato spotted wilt virus, a blue fluorescence is found first as a halo in the mesophyll cells surrounding the lesions. Later the vascular tissue becomes fluorescent and still later scopoletin is accumulated in the roots (Best

TABLE 1. *The Occurrence of Scopoletin in Plants*

Family and species	Portion of plant	Author
Gramineae		
Avena sativa L.	Roots	Goodwin & Kavanagh (this paper)
Rosaceae		
Prunus serotina Ehrh.	Bark[a]	Power & Moore (1909)
Rutaceae		
Chalcas exotica (L.) Millsp. (= *Murraya exotica* L.)	Blossoms[b]	Blas (1868)
Loganiaceae		
Gelsemium sempervirens Ait. f.	Roots	Coblentz (1897), Schmidt (1898), Moore (1911), Seka & Kallir (1931)
Convolvulaceae		
Convolvulus scammonia L.	Roots	Power & Rogerson (1912)
Ipomoea Purga Hayne	Roots	Power & Rogerson (1910)
Solanaceae		
Atropa Belladonna L.	Whole plant extracted	Kunz (1885)
Fabiana imbricata R. & P.	Leaves and twigs[a]	Kunz-Krause (1899), Edwards & Rogerson (1927)
Nicotiana Tabacum L.	Root, stem, leaf	Best (1944, 1948)
Scopolia japonica Max.	Roots[a]	Eijkman (1884), Henschke (1888)
Solanum tuberosum L.	Tubers	Andreae (1948)
Compositae		
Artemisia spp.		Sample from Wellcome Research Institution

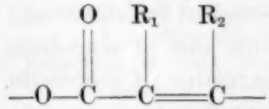
[a] Also obtained as the glucoside.
[b] Obtained as the glucoside.

1948). Scopoletin is found in potato tubers infected with leaf roll virus (Andreae 1948), the fluorescence occurring in cells adjacent to necrotic phloem tissue (Sanford & Grimble 1944). Diseased tubers can be quickly selected under ultraviolet light by examining the cut surfaces for fluorescence. The use of this technique in sorting seed potatoes may prove useful in reducing the incidence of infection by leaf roll and other types of virus (McLean & Kreutzer 1944).

From a quantitative standpoint it is interesting to note that scopoletin has been extracted in the following amounts: from *Fabiana imbricata* twigs, 1.4 mg. per gram of dried twigs (Edwards & Rogerson 1927); from *Gelsemium sempervirens* roots, 0.12 mg. per gram wet weight (Seka &

Kallir 1931); from diseased potato tubers, circa 0.01 mg. per gram wet weight (Andreae 1948); from *Avena sativa* roots, 0.01 mg. per gram wet weight.

Coumarin and its derivatives are among the compounds known as unsaturated lactones, which are characterized by the presence of the structure



within the molecule. These unsaturated lactones have been shown to have physiological activity in many different kinds of organisms (Veldstra & Havinga 1945). In higher plants and bacteria they have been found to inhibit growth, and several of them are antibiotic substances. Parasorbic acid, one of the simpler unsaturated lactones, for instance, inhibits germination of seeds (Kuhn *et al.* 1943) and cell enlargement in the *Avena* coleoptile (Larsen 1947), while protoanemonin inhibits the growth of roots and slows down their metabolism (Goddard 1948). Some of the coumarins are still more effective inhibitors. Coumarin, aesculetin, and daphnetin inhibit seed germination (Cameron 1910; Sigmund 1914; Nutile 1945), while coumarin inhibits elongation (Audus 1948) and mitosis (Cornman 1946, 1947) in roots. Experiments now in progress indicate that scopoletin also inhibits the growth of roots at concentrations as low as 10^{-5} molar.

Unsaturated lactones have been shown to react specifically with compounds containing the sulfhydryl (–SH) radical (Cavallito & Bailey 1944; Cavallito & Haskell 1945). Since the presence of the sulfhydryl group has been found to be essential to cell proliferation (Hammett & Chapman 1938), the growth-inhibiting effects of the unsaturated lactones may well be due to the inactivation of sulfhydryl-containing enzyme systems. Thimann and Bonner (1949) have shown that the inhibiting action of protoanemonin and coumarin on growth is prevented by the presence of 2, 3-dimercaptopropanol, a compound known to protect sulfhydryl groups from inactivating substances. The unsaturated lactones are frequently found in physiologically significant concentrations within the tissues. Whether scopoletin acts as a natural growth regulator is not known, but the fact that it is present in mature portions of *Avena* roots at a concentration in excess of 5×10^{-5} molar, a concentration at least twenty times that found in the growing tip (apical three millimeters), is suggestive.

SUMMARY

The isolation and identification of one of the blue-fluorescing compounds in *Avena sativa* roots as scopoletin (6-methoxy-7-hydroxy coumarin) is reported. This substance is present in mature portions of five-day-old roots in concentrations in excess of 10 ppm. From three to 18 millimeters behind

the root apex, the amount of scopoletin per millimeter of root was found to lie between 0.75×10^{-9} and 1.5×10^{-9} gram. In the apical three millimeters, which includes the meristem and the region of cell elongation, the amount of scopoletin was too small to measure, but was certainly less than 0.08×10^{-9} gram per millimeter.

Published studies on the growth-inhibiting properties of coumarin and its derivatives are cited with reference to the possible role of scopoletin within the plant. In this regard the very low concentration of scopoletin in the actively growing portion of the root may be significant.

In this study, fluorescence has proved to be a very useful tool in locating, identifying and determining the amounts of scopoletin which are present in the plant.

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NOTES ON THE CYTOLOGY OF SOME LYCOPSIDS

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A number of Wisconsin Lycopsids were investigated to determine somatic chromosome numbers and study peculiarities in somatic division.

Marquette (1907) first described spindle-formation as related to the plastids present in the young leaves of *Isoetes lacustris* L. (*Isoetes macrospora* Dur.) The presence of a single "starch body" (plastid) in each cell, the proximity of that "starch body" to the nucleus, its elongation and division before the prophases, the major role the daughter plastids play in spindle formation, and the persistence of the daughter "starch body" in the daughter cell were illustrated and described; its centrosome-like function was pointed out, and the name "polar structure" was suggested. Marquette concluded that " . . . it might possibly be assumed that in *Isoetes* we have a transition from a cell structure with well defined central bodies, as found in some algae and fungi, to a cell structure apparently without central bodies or anything corresponding to them as found in the spermatophytes."

Ma (1928) studied the chloroplasts of *Isoetes melanopoda* Gay & Dur. with reference to content and function during nuclear division. She found that the single plastid present in an embryonic cell lies in close contact with the nucleus and divides into two plastids which take polar positions during nuclear division. In some cells whose nuclei show no evidence of impending division two plastids were sometimes present, and in older cells and those exposed to light the plastids were more numerous.

Ekambaram and Venkatanathan (1933) discuss sporangium development and meiosis in the macro- and microsporangia of *Isoetes coromandelina* L. They found that in the heterotypic prophases " . . . the first visible change initiating division in the mother-cells is the appearance in the cytoplasm of a body very close to the nucleus. This is clearly made out when it forms a deep crescent adjoining the nucleus. It has a clear outline and appears to contain some dark staining granules of various sizes and other minute grains whose nature could not be made out with the methods of staining employed."

This structure referred to as a "polar body" divides to form four bodies which become arranged tetrahedrally near the periphery of the spore-mother cell. The relation of these four bodies to the formation of a tetrapolar spindle which becomes bipolar, their behavior during the division of nucleus and cell, the gradual decrease in size of the "polar body" present in each

young spore, and its ultimate disappearance in the fully developed spore are described. Similar bodies are met with also in the dividing vegetative cells. The chromosome count of *I. coromandelina* is given as $n = 16$.

Yuasa (1935) studying microsporogenesis in *Isoetes japonica*, finds the haploid number to be 33, and concludes that the species is a hexaploid. In his summary no mention is made of polar structures or of plastids, nor do the illustrations show them.

Ekstrand (1920) found the haploid chromosome number in *Isoetes echinospora* to be 11, the diploid 22. During the metaphases the spindle was sometimes seen to have two sharp-pointed poles, but centrosome-like bodies were not observed.

Materials and Methods. Root-tips, stem-tips, and strobili of *Lycopodium annotinum* L., *L. annotinum* var. *acrifolium* Fernald, *L. obscurum* L., *L. clavatum* L., *L. lucidulum* Michx., *L. inundatum* L., *L. Selago* var. *patens* (Beauv.) Desv., *L. complanatum* L., *Selaginella rupestris* (L.) Spring., *Isoetes macrospora* Dur., and *I. muricata* var. *Braunii* (Dur.) Reed, were collected in one or more of the Wisconsin counties: Vilas, Washburn, Langlade, and Sauk.

Material was fixed at the time of collection in Flemming's medium solution or in Randolph's or Belling's modification of Navashin's fixative. A cedar–anilin oil series or a terpineol series was used to avoid higher alcohols and chloroform.

Stains used were Heidenhain's iron-alum haematoxylin, Flemming's triple stain, and Crystal violet–iodine with picric acid as recommended by Smith (1934). Plants from which fixations were made were preserved as herbarium specimens.

ISOETES. A cell of any tissue in *Isoetes muricata* var. *Braunii* not too far differentiated to possess plastids at all, and if not actually dividing or preparing to divide, contains in general a single plastid (fig. 1). The description that follows applies particularly to cells of the root-tip.

A plastid is ellipsoidal to irregular in shape, about one-half to one-fourth the size of the nucleus, to which it is appressed. In the plane of contact the nuclear membrane may be flattened. The outer boundary of the plastid is membrane-like in appearance, often strikingly definite.

In preparations treated with iodine it usually can be seen that each plastid contains starch grains staining blue or brown; in crystal violet–iodine the grains are stained bright blue. The number in a plastid varies from two or three to fifteen or twenty. The starch grains are nearly spherical, ovoid, or disc-like. In the latter case they appear from oval to circular in surface view. They are not laminated; some possess a dark-staining cen-

tral hilum; others have a light-staining hilum and a darker margin. They vary greatly in size, the largest being five or six times the volume of the smallest. Plastids are present that contain very little starch, and others that include no starch. A plastid containing no starch can be distinguished only by careful observation and light-adjustment. The tiny starch grains when present can be shown to be optically active when between crossed polarizing screens.

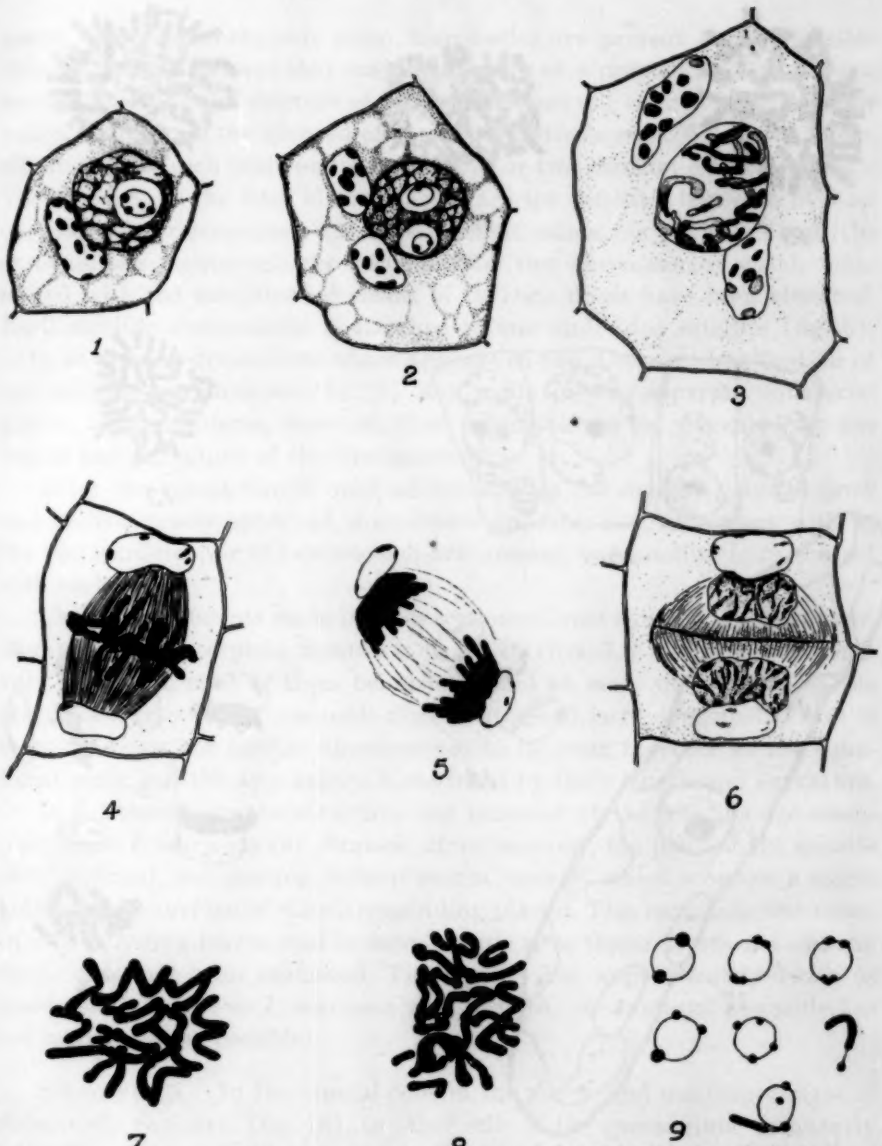
The single plastid present in an elongating and maturing cell may divide to form two or more daughter plastids (fig. 2). In such a case the plastids are commonly grouped about the nucleus; sometimes they appear in other parts of the cell. In maturing or mature cells plastids may be absent, or at least not detectable. These plastids are evidently similar to those first noted in young leaf cells of *I. macrospora* by Marquette (1907); he referred to them as masses of starch grains, each group apparently surrounded by a membrane.

Before the beginning of the prophases, the single plastid present in a meristematic cell elongates and finally divides; both ellipsoidal daughter halves then migrate until the two lie at opposite sides of the nucleus in contact with or close to the nuclear membrane (fig. 3). As the nuclear membrane disappears the plastids separate slightly and spindle fibers are seen to meet them along nearly half their proximal surfaces. The broad poles of the spindle are particularly evident during the equatorial plate stage (fig. 4) and the metaphases. When the plastids contain no visible starch they appear as vacuole-like structures at the poles of the spindle. However in some instances they may still be demonstrated because of their optically active contents.

During the late anaphases the shape of the chromosome group may be affected by the presence of the plastid; and the telophase daughter nucleus, in immediate contact with the plastid, is sometimes almost crescent-shaped (fig. 6). Nuclear division is followed by the formation of the cell plate which brings about the division of the cell (fig. 6). This history is similar to that reported by Marquette for the leaf cells of *I. macrospora*.

The nucleus of a resting meristematic cell occupies a central position and characteristically possesses one or two nucleoli which are left translucent by the crystal violet–iodine stain. Without exception, in all preparations made from variously fixed material, stained either with crystal violet–iodine or with the triple stain, a clear space is present about each nucleolus (figs. 1, 2).

In contact with the nucleolus, if a single one is present, two, three, or four highly chromatic bodies can be seen (fig. 9). They stand out sharply on the surface of the faintly stained nucleolus because of their chromatic nature. When two such bodies are present, they are spherical, slightly elon-



Isoetes muricata var. *Braunii;* cells in root-tip meristem. FIG. 1. Nucleus in very early prophase; single starch-containing plastid. FIG. 2. Nucleus in early prophase; two daughter plastids. FIG. 3. Late prophase; daughter plastids at opposite sides of nucleus. FIG. 4. Equatorial plate; plastids at spindle poles. FIG. 5. Plastids in contact with anaphase chromosome groups. FIG. 6. Plastids in contact with telophase nuclei. FIGS. 7, 8. Equatorial plates; showing 24 and 25 chromosomes. FIG. 9, *a–e*. Satellites in contact with nucleolus. *f*. Chromosome with satellite. *g*. Nucleolus with satellite chromosome.

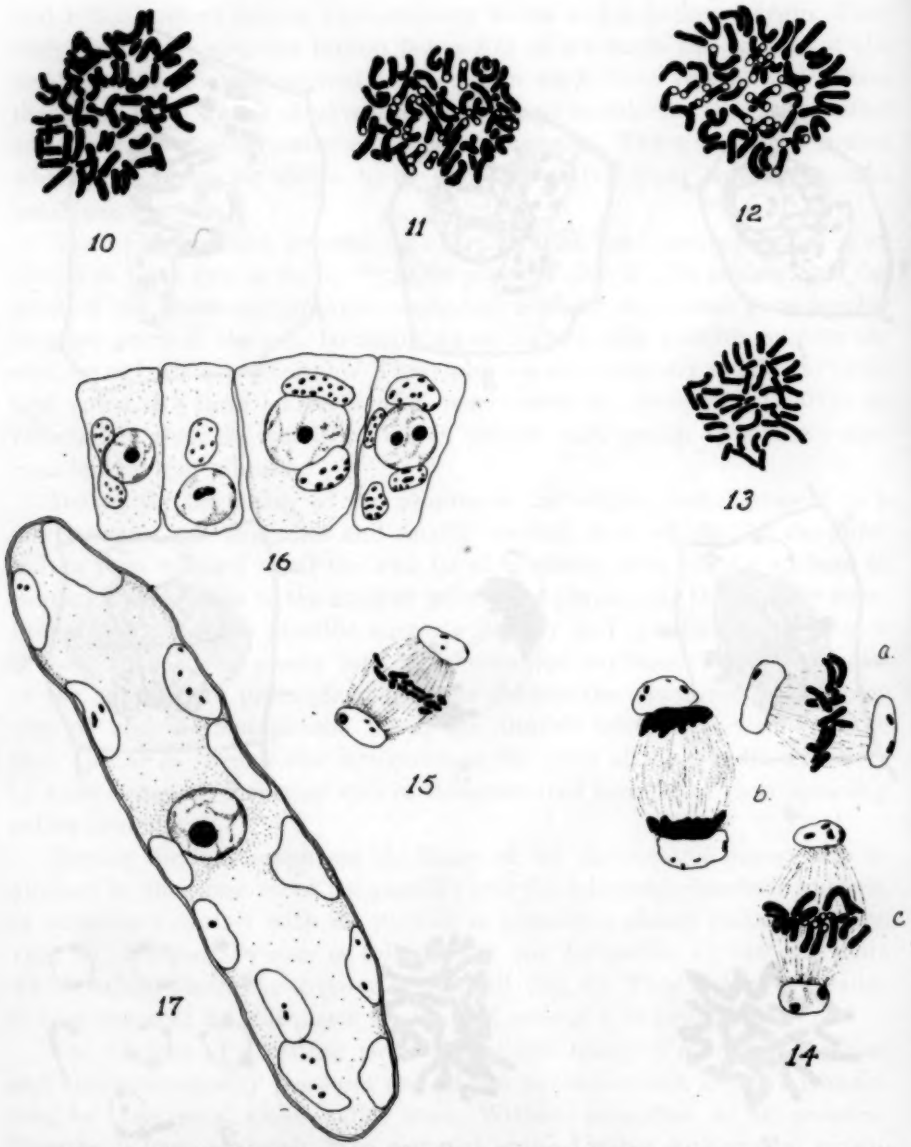


FIG. 10. Equatorial plate of *L. clavatum* var. showing 60 chromosomes. FIG. 11. Equatorial plate of *L. obscurum* showing 50 chromosomes. FIG. 12. Equatorial plate of *L. annotinum* showing 50 chromosomes. FIG. 13. Equatorial plate of *L. complanatum* var. *elongatum;* 40 chromosomes. FIG. 14, *a, c.* Equatorial plates on *L. complanatum* var. *elongatum* showing plastids at the spindle poles. *b.* Plastids in contact with anaphase chromosome groups of *L. complanatum* var. *elongatum.* FIG. 15. Exceptionally large figure in *Selaginella rupestris* showing plastids at spindle poles. FIG. 16. Plastids containing red-staining granules in tapetal cells of *S. rupestris.* FIG. 17. Chloroplasts containing red-staining granules in leaf cell of *S. rupestris.*

gated, or dumbbell-shaped; when four bodies are present each is smaller than in the case of two; they may lie in pairs or separated by variable distances. The different degrees of separation observed suggest that the four bodies result from the division of the two sometimes seen. When two nucleoli are present each bears on its surface one or two chromatic bodies (fig. 2). These bodies, as the later history shows, are the satellites borne on at least one pair of chromosomes. When the nucleus enters the prophases and the chromosomes become clearly recognizable, two chromosomes visibly connected with the satellites by means of delicate fibers have been observed. Each satellite chromosome is attached to one undivided satellite (fig. 9), or to an already divided one which appears as two. Certain identification of one satellite chromosome (fig. 9) was made in two separate equatorial plates; in most plates, however, their identification is prevented by the length and curvature of the chromosomes.

After the completion of nuclear division, as the daughter nuclei grow and become nearly spherical, a nucleolus appears, and, in contact with it, the two satellites; or if two nucleoli are present, one satellite is in contact with each.

Chromosome counts made in cross sections of root tips of *I. muricata* var. *Braunii* show the diploid number to be 24–26 (figs. 7, 8). The chromosomes vary in length, most of them being long, and on some the position of the centromere to which the spindle fiber is attached is recognizable. There is some tendency for similar chromosomes to lie near together in the equatorial plate, but the appearance is confused by their length and curvature.

In *I. macrospora* the structure and behavior of the plastids are essentially as in *I. muricata* var. *Braunii*. Here, however, the poles of the spindle are not broad, but sharply defined points, each of which occupies a slight hollow in the surface of the corresponding plastid. This condition was noted in cells of young leaves, and in meristematic stem tissue. Roots of *I. macrospora* have not been examined. This species has approximately twice as many chromosomes as *I. muricata* var. *Braunii;* the material available has not made a count possible.

SELAGINELLA. In the tapetal cells in the micro- and macrosporangia of *Selaginella rupestris* (fig. 16), in other cells of the sporangium, frequently in embryonic and elongating stem cells, and in leaf and ligule primordia, leucoplasts are present which differ from the chloroplasts in cells of the stem and leaves in size and in staining reaction. Each cell contains one or two, occasionally three or four leucoplasts. These bodies are from spherical to ellipsoidal in shape and usually smaller than the nucleus, about 5 to 6 μ in diameter; they usually lie in contact with or close to the nucleus, but sometimes appear in other parts of the cell. A leucoplast has a distinct

outer margin, suggesting the presence of a limiting membrane. Within the leucoplast, when stained with the triple stain, are seen from 2 or 3 to 15 or 20 small red granules. The granules vary somewhat in shape, usually being spherical or ellipsoidal, sometimes more elongated. All are of approximately the same size, about 1 μ in diameter; they do not turn blue in the presence of iodine and are optically inactive.

Ma (1930) has described the chloroplasts of *Selaginella* with special reference to those in the leaf cells of *S. apoda* . . . "in which a single plastid is found in most cells. . . . By the time the leaves have reached a length of 0.2 mm., spindle-shaped red-staining bodies are clearly recognizable in the plastids . . . as the plastids enlarge with the increase in size of the cells, the bodies, which stain red with the triple stain, also increase in size." Ma noted blue-staining starch grains also in the same plastids, the starch grains being of the same shape and size as the red-staining bodies. In some plastids, bodies were present whose staining reaction was intermediate between that of the red bodies and the starch grains. The red bodies gave a positive protein reaction with Millon's reagent, since it appeared that these red-staining bodies in the plastids of *S. apoda* may be transformed into starch grains, it was suggested that they are similar in nature and function to the pyrenoid bodies of *Anthoceros* and *Notothylas* (McAllister 1926):

The chloroplasts of *Selaginella rupestris* (fig. 17), most easily studied in the leaves, are ellipsoidal. Each contains from one to five or six spherical red-staining bodies similar to those in the leucoplasts. None of my preparations show anything in either chloroplast or leucoplast that would suggest a transition from the red-staining bodies to blue-staining starch grains. Each of the many chloroplasts in a leaf cell (fig. 17) is about 3 μ in width and 6 μ in length. The red granules vary in size from about 0.5 to 1 μ, whereas starch grains present in the plastids vary from about 0.5 to 3.5 μ in diameter. From the staining reactions of the isotropic granules in the chloroplasts it seems evident they are not starch; it is possible that they are proteinaceous in nature.

Division figures were noted in cells of *S. rupestris* in which one plastid occupied each pole of the spindle (fig. 15), but these figures were too few in number to justify a statement on the behavior of the plastid during division. The diploid chromosome count is more than 12.

LYCOPODIUM. In young sporangia of *Lycopodium complanatum* var. *elongatum* in which premeiotic nuclear divisions are occurring, sporogenous cells not dividing or preparing to divide contain, as a rule, a single leucoplast. The plastid is elongated, appressed to the nucleus, and contains variable numbers of starch grains. The grains, which turn blue in the presence

of iodine and stain bright blue with crystal violet–iodine, are similar in shape to those described in *Isoetes muricata* var. *Braunii*, and number commonly from 2 or 3 to 10 or 15 in each plastid. Some plastids contain very little starch; in some starch is entirely absent. The latter plastids appear like vacuoles. The plastid in a cell whose nucleus is in the very early prophases is elongated; somewhat later it divides to form daughter plastids the starch content of each of which is approximately half that of the original plastid. The two daughter plastids come to lie on opposite sides of the nucleus and in close contact with the nuclear membrane. Some cells have been seen which contained more than two plastids, two of which lie at opposite sides of the nucleus, the others lie near the plasma membrane.

At the equatorial-plate stage, at which time the typical broad poles of the spindle can best be seen (fig. 14, *a*, *c*), in preparations stained with crystal violet–iodine, starch grains are usually visible at each pole of the spindle; careful observation shows that they lie within a plastid apparently bounded by a membrane. During the metaphases, anaphases (fig. 14 b), and telophases likewise, a starch-containing plastid can usually be seen at each broad pole of the spindle. If starch is absent, the plastid at each pole appears much like a vacuole. Thus it is evident that, as in the embryonic root-tip cells of *Isoetes muricata* var. *Braunii* and in embryonic leaf and stem cells of *I. macrospora*, the single plastid present in a sporogenous cell of *Lycopodium complanatum* var. *elongatum* divides to form two daughter plastids which function in the formation of the spindle and which occupy the spindle poles during nuclear division, persisting during the telophases and in the daughter cells.

Chromosome counts made in root tips showed the diploid number to be 40 (fig. 13). The chromosomes of this species as seen in the equatorial plate are all rather short.

Premeiotic nuclear divisions in sporogenous tissue of *L. obscurum* show in some cases a plastid at each spindle pole. This condition was noted in a few equatorial-plate figures and in a few anaphases. In all these cases starch grains were present in each plastid. However, in the material studied so little starch was present that plastid identification was often uncertain.

Chromosome counts were made from cross sections of rhizome tips and from sporangia. The diploid chromosome number is about 50 (fig. 11). The chromosomes vary greatly in length, some being short but the majority rather long. The length of the chromosomes is not so evident in polar views as in lateral views of equatorial plates. In the latter, the long arms of some of the chromosomes reach almost to one or the other spindle pole.

In root tips of *L. annotinum*, each embryonic or elongating cell possesses from one to 8 or 10 plastids. A plastid is ellipsoidal to spherical in shape and contains in addition to starch grains from 2 or 3 to 15 or 20 granules

which vary little in size. These granules are similar to those present in *Selaginella rupestris;* like those, they take a red color in the triple stain. The plastids usually lie appressed to the nucleus, and if sufficiently numerous they may completely surround the nucleus. Staining reactions supply no evidence that the red-staining granules change to starch. The starch grains are present in the same plastids with the red-staining granules, are from 4 to 6 times their diameter, and are blue with the triple stain. Whether or not the red granules are in any way related to starch formation is uncertain.

In preparations stained with iron-alum haematoxylin the plastids are clearly visible and the included starch grains are from gray to black. No structures appear that correspond to the red-staining granules, so conspicuous with the triple stain. In haematoxylin-stained preparations the membrane-like outer boundary of the plastid is readily recognizable, the starch grains lying in close contact with it.

The chromosomes of this species vary from 3 to 5 μ in length; their curvature and crowding make counting difficult. The diploid number is about 50 (fig. 12).

In root-tip preparations of *L. clavatum* stained with iron-alum haematoxylin, plastids, one or two in each cell, appear similar to those of *L. annotinum*. They are in contact with the nuclear membrane and contain from gray to black starch grains similar to those of *L. annotinum*. In equatorial-plate, metaphase, anaphase, and telophase stages, the starch grains are visible at spindle poles, and often it is evident that the grains lie within a plastid. Here, then, as in *L. complanatum* var. *elongatum*, it appears that the plastids function in the formation of the achromatic figure and that they occupy the poles of the spindle during nuclear division.

The diploid chromosome count is about 60 (fig. 10). Counting is made difficult, as in *L. annotinum*, by the curvature and crowding of the chromosomes.

The chromosome numbers of *L. inundatum* and *L. lucidulum*, studied in the root-tips, seem to be approximately the same as *L. clavatum*. Similarities to *L. clavatum* appears also in the sizes and shapes of the chromosomes. Mitotic figures were too crowded for a satisfactory count of the chromosomes. In root-tip cells of *L. inundatum* and *L. lucidulum*, starch-containing plastids were observed apparently similar to those of *L. complanatum* var. *elongatum* and *L. obscurum*.

Conclusion. If a single plastid present in any embryonic cell divides to form two daughter plastids which function in the formation of an achromatic figure, occupy the spindle poles during nuclear division, and persist in the daughter-cells, the entire sequence of events may be spoken of as

plastid-polarity. Somatic and meiotic plastid-polarity are convenient terms to designate in which type of tissue this polarity is exhibited.

Similar somatic plastid-polarity has been shown to exist in *Isoetes macrospora* (Marquette 1907), *I. melanopoda* (Ma 1930), *I. coromandelina* (Ekambaram 1933), and *I. muricata* var. *Braunii* (herein reported). Meiotic plastid-polarity is shown to be present in *I. coromandelina* (Ekambaram 1933). The function of plastids in spindle-formation and their presence at the poles of the spindle during nuclear division in these four species suggest the possibility of their occurrence and like function in other species of *Isoetes*.

In *Selaginella rupestris* a few figures were seen in which a plastid was present at each pole of the spindle.

TABLE 1. *Chromosome Numbers in the Lycopsida*

Species	Number reported		Author
	n	*2n*	
Isoetes coromandelina	16	.....	Ekambaram & Venkatanathan 1933
I. echinospora	11	.....	Ekstrand 1920
I. asiatica	.....	22	Takamine 1921
I. japonica	.....	43–45	Takamine 1921
I. japonica	33	.....	Yuasa 1935
I. muricata var. *Braunii*	.....	24–26	Dunlop
Selanginella Vogelii	.....	16	Heitz 1925
S. Ouvrardii	.....	20–22	Heitz 1925
S. Martensii	.....	52–57	Heitz 1925
S. grandis	.....	16–17	Heitz 1925
S. emiliana	8	.....	Denke 1902
S. serpens	8	.....	Denke 1902
S. rupestris	.....	12+	Dunlop
Lycopodium clavatum	14	.....	Baranov 1925
L. clavatum var.	.....	ca. 60	Dunlop
L. complanatum var. *elongatum*	.....	40	Dunlop
L. annotinum	.....	ca. 50	Dunlop
L. obscurum	.....	ca. 50	Dunlop

Somatic plastid-polarity has been described above in the premeiotic nuclear divisions of *Lycopodium complanatum* var. *elongatum;* the condition is similar to that in Isoetes. In *L. obscurum* premeiotic figures were seen that strongly suggest polarity. In embryonic root cells of *L. annotinum*, starch-containing plastids were seen at the spindle poles in division figures.

The occurrence of somatic plastid-polarity in both *Isoetes* and *Lycopodium*, and perhaps also in *Selaginella* is significant.

Starch-containing plastids were observed in all species studied. Plastids containing granules colored red in the triple stain were seen only in *Selaginella rupestris* and *Lycopodium clavatum*. The nature of the red-staining granules is not clear. Similar granules present in *Selaginella apoda* were thought by Ma (1930) to have a pyrenoid-like function.

Chromosome size within the Lycopsida (in those species studied) varies from the extremely small and short chromosomes of *Selaginella rupestris*, 1 μ or less in length, to the large, long chromosomes of *Isoetes muricata* var. *Braunii* which may be 0.5–1 μ in thickness and 7 or 8 μ long. Chromosomes of *Lycopodium complanatum* var. *elongatum* are about 0.5 μ in thickness and 1–3 μ long. Those of the other species of *Lycopodium* studied are intermediate in length between those of *L. complanatum* var. *elongatum* and *Isoetes muricata* var. *Braunii*, and are from 1 to 1.5 μ in thickness. Satellite chromosomes were seen in preparations of *Isoetes muricata* var. *Braunii*, but not in the other species studied.

SUMMARY

In *Isoetes muricata* var. *Braunii* plastids function in the formation of the achromatic figure and occupy the spindle poles during nuclear divisions in the meristem of the root tip.

Isoetes muricata var. *Braunii* possesses at least one pair of satellite chromosomes.

Red-staining granules of uncertain function are present in chloroplasts and leucoplasts of *Selaginella rupestris*. Similar bodies were observed in leucoplasts in root cells of *Lycopodium annotinum*.

Plastid behavior during premeiotic nuclear division in *Lycopodium complanatum* var. *elongatum* is similar to that during nuclear division in *Isoetes muricata* var. *Braunii*. In *Lycopodium obscurum* and *L. clavatum* evidence of plastid behavior like that in *Isoetes muricata* var. *Braunii* is strong.

Chromosome size and number in some Wisconsin species of the Lycopsida have been determined.

The author is indebted to Dr. Charles E. Allen for his constructive criticism.

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POSTGLACIAL FORESTS IN WEST CENTRAL ALBERTA, CANADA[1]

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Peat sections were obtained from four bogs along the Edmonton-Jasper highway in west central Alberta. These bogs lie at distances of about 30, 55, 85, and 125 miles west of Edmonton. More specifically they are located about 4 miles north of Duffield, 4 miles east of Entwhistle, near the town of McKay, and about 5 miles west of Edson, respectively. Hereafter they will be referred to as the Duffield, Entwhistle, McKay, and Edson sections or bogs. All sites lie within the westernmost border of the Keewatin drift. Although the Cordilleran glaciers descended the east slope of the Rocky Mountains in this latitude, the relationships between the two ice sheets and the borders of their drifts are uncertain. It is evident, however, that the Keewatin ice was more extensive in this region, because the Cordilleran glaciers were probably limited by the relatively low precipitation on the east slope of the mountains. One stage of the Cordilleran glaciers descended the eastern flanks of the Rockies to points 50 miles east of the mountains in the vicinity of Calgary, and still farther east in the latitude of Edmonton (Flint 1947). During one or more stages the Cordilleran and Keewatin ice may have coalesced, but there is no evidence that their fronts were joined during the Late Wisconsin (Mankato) stage in west central Alberta. It seems probable that the last glacier in the region was of Keewatin origin. Keewatin boulders have been noted as far west as Edson, although no end moraines have been observed in this area (Erdtman & Lewis 1931). The eastern border of the Altamont moraine, which is dated as terminal Late Wisconsin, lies about 75 miles east of Edmonton (Bretz 1943). It is considered to be equivalent to the Mankato maximum of the Minnesota-Iowa region. West of the Altamont moraine in the general latitude of Edmonton are older Keewatin moraines. The westernmost and oldest of these is the Duffield moraine, whose eastern border lies about 25 miles west of Edmonton. Although the Duffield moraine is older than the Altamont, its topography is much more youthful than the Early Wisconsin (Iowan) drift in Montana (Bretz 1943). In a previous paper (Hansen 1949) it was suggested that the Duffield was equivalent to the Tazewell or Cary of the eastern ice, although geologists have not recog-

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nized an intermediate Wisconsin stage for the western ice (Antevs 1945). Lying between the Duffield and the Altamont moraines are the Buffalo Lake and Viking moraines which are younger than the former but older than the latter (Bretz 1943). If the uppermost drift for 200 miles west of the Altamont is of Keewatin origin, but older than the Altamont, there must have been an ice-free area which may have been forested during the Late Wisconsin glaciation. The possibility that bogs lying between Edmonton and the westernmost border of the Keewatin ice on the east flank of the Rockies are pre-Late-Wisconsin is supported by the fact that the Keewatin ice retreated downslope so that the meltwater was ponded against the ice and drained laterally. This may have eliminated a long period of physiographic instability which would have hindered the formation of permanent basins in which hydrarch succession took place. There is greater possibility that meltwater from the Cordilleran glacier, flowing eastward, may have destroyed or buried sites of pre-Late Wisconsin bogs, or on the other hand prevented hydrarch succession and the deposition of pollen-bearing sediments during the Late Wisconsin stage near the mountains. The pollen record in peat sections west of the Altamont moraine, however, suggests that forests did exist in this ice-free area at least during the latter part of the Late Wisconsin.

Characteristics of the Bogs. All the bogs are in the mature stage with climax forest trees on their surfaces. Sphagnum moss and Labrador tea (*Ledum groenlandicum*) and other species of ericaceous plants cover the bogs, with local areas of sedges and other hydrophytic species. Trees on the muskegs include black spruce (*Picea mariana*), tamarack (*Larix laricina*), and lodgepole pine (*Pinus contorta*), the latter being absent on the Duffield bog. Bog birch (*B. glandulosa*) is very common, and in some areas is more abundant than Labrador tea. Other muskegs in the area were noted that supported dense forests of large black spruce and some white spruce (*Picea glauca*), suggesting that hydrarch succession had been terminated for some time, perhaps owing to the age of the bog or the shallowness of the depression. The depth of the bogs in the area of sampling is as follows: the Duffield, 3 m.; the Entwhistle, 6.5 m.; the McKay, 1 m.; and the Edson, 7.0 m. In the Duffield and Edson sections, almost a meter of clay at the bottom was devoid of pollen. In the Entwhistle section, volcanic glass was identified at 3 and 3.2 m. and at 0.6 m. In the Edson section, glass was noted from 4.2 to 4.6 m. and at the surface. The source of the ash is not certain, but some of it may have come from Glacier Peak in north central Washington, while some may have been derived from the west, possibly the Coast Range of British Columbia. It seems probable that the glass near the surface came from the eruption of Mt. Katmai in Alaska in 1912. This is supported by the occurrence of volcanic glass in the upper levels of peat sections from many bogs

along the Alaska highway in northeastern British Columbia. It is possible that the glass at the 3 m. level in the Entwhistle section is synchronous with the 4.4 m. horizon in the Edson column, as suggested by their respective trends of forest succession.

The relative depths of the bogs provide no criteria with respect to their relative ages. If the area in which the bogs are located was not influenced by the Cordilleran ice of the Late Wisconsin glaciation, then it could be assumed that the westernmost was the oldest. On the other hand, if the Cordilleran ice succeeded the Keewatin, particularly in the western portion, the Edson bog might be considered the youngest. This possibility is supported by its formation in a river valley which carries water from the mountains to the west. During melting of the Cordilleran ice, the valley probably carried considerable more water than it does at present, thus preventing hydrarch succession until the stream had subsided. The relative stratigraphic position of the volcanic glass and the pollen profiles suggests that the Edson section is younger than the other thick section near Entwhistle.

In preparation of the peat for pollen analysis, the potassium hydrate method was used. Pollen is abundant except in the inorganic, clay sediments underlying the organic peat, which suggests absence of forests in the region when these accumulated. From 100 to 200 pollen grains of forest trees were identified at each level. An attempt was made to separate lodgepole from jack pine and white spruce from black spruce by their pollen size ranges. In interpreting the pollen profiles, however, it was found to be infeasible to use the species separately, because of the many sharp fluctuations from level to level, which could not be ascribed to any logical cause.

Forests of the Region. In the general vicinity of Edmonton occurs the meeting place of three climax vegetation formations of North America. These are the boreal forest, the Cordilleran forest, and the grasslands (Lewis et al. 1928). Lying between the grasslands and the boreal forest to the north and the Cordilleran forest to the west is a transition zone known as the Parkland because of the scattered groves of trees, consisting largely of aspen (*Populus tremuloides*) in the southern part, and aspen, white spruce, white birch, and balsam poplar (*P. tacamahacca*) in the northern part. Moss (1932) divides the Parkland into a northern Poplar area and retains the name Parkland for only the southern third. He also divides the grassland into a northern and southern prairie. The occurrence of black and brown soils in the Poplar area and Parkland as well as podsolized prairie soils in the southern fringe of the boreal forests suggests greater extension of grassland in the past, perhaps during the warm, dry maximum between 8,000 and 4,000 years ago (Hansen 1947, 1949).

The arborescent species of the boreal forest are black and white spruce,

balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*), tamarack, aspen, balsam poplar, and white birch. White spruce and its varieties are the most abundant, widespread, and permanent, and in the absence of fire and cutting they become the climax dominants except in the northern part of their range where black spruce and tamarack invade higher ground and act like climax dominants, and in certain local areas. Balsam fir also may be considered a climax species, although it is not so abundant as white spruce, and probably does not range west of north central Alberta (Halliday & Brown 1943). Jack pine is subclimax on burns and assumes a climax role on sandy areas in the white spruce forest. Lodgepole pine replaces jack pine in the boreal forest in western Alberta, British Columbia, and the Yukon. Their ranges overlap within 20 miles west and northwest of Edmonton and throughout the Peace River and Lesser Slave Lake regions.[2] Unlike jack pine, lodgepole often invades mature bogs with black spruce, tamarack, and white birch. Aspen, poplar, and white birch form a fire subclimax associes throughout the boreal forest of Alberta and British Columbia, with either jack pine or lodgepole pine, depending upon the location and soil.

The Cordilleran forest is included in the Petran subalpine and Petran montane forests (Weaver & Clements 1938), which extend along the crest and the upper slopes of the Rockies. It extends eastward from the mountains, forming a narrowing wedge reaching almost to the center of the province of Alberta. The arborescent species of the Cordilleran forest in Alberta include subalpine fir (*Abies lasiocarpa*), which replaces balsam fir of the boreal forest, Engelmann spruce (*Picea engelmanni*), lodgepole pine, and aspen. Whitebark pine (*Pinus albicaulis*), limber pine (*P. flexilis*), and Douglas fir (*Pseudotsuga taxifolia*) occur in the southern part of the Cordilleran forest in the mountains. White spruce ranges throughout the Cordilleran forest southward into Montana (Munns 1938), while both tamarack and black spruce are common on muskegs in the more northern part. Aspen, poplar, and white birch also occur abundantly in the Cordilleran forest, where aspen is the most abundant because of its invasion of burns. At present, however, the predominant forest species in the Cordilleran forest of Alberta is probably lodgepole, which occupies burns and other edaphically disturbed areas, either initially or with aspen. Lodgepole also becomes progressively more abundant westward toward the Rockies.

The easternmost bog of this study is located in the western part of the Poplar area, while the other three lie within the Cordilleran forest. Lodgepole is not common in the vicinity of the easternmost bog, but it becomes progressively more abundant westward. Grassland openings are common.

Postglacial Forest Succession. The pollen profiles indicate that pine and spruce have been the principal species in the region throughout the time

[2] Information from Professor E. H. Moss, University of Alberta.

represented by the sedimentary columns (figs. 1, 2, 3, 4). At most levels, they constitute more than 90 per cent of the total tree pollen. It is unfortunate that aspen pollen is so poorly preserved, because this species undoubtedly played an important part in postglacial forest succession, as is suggested by its present proportions in the Cordilleran and boreal forests. Its invasion of

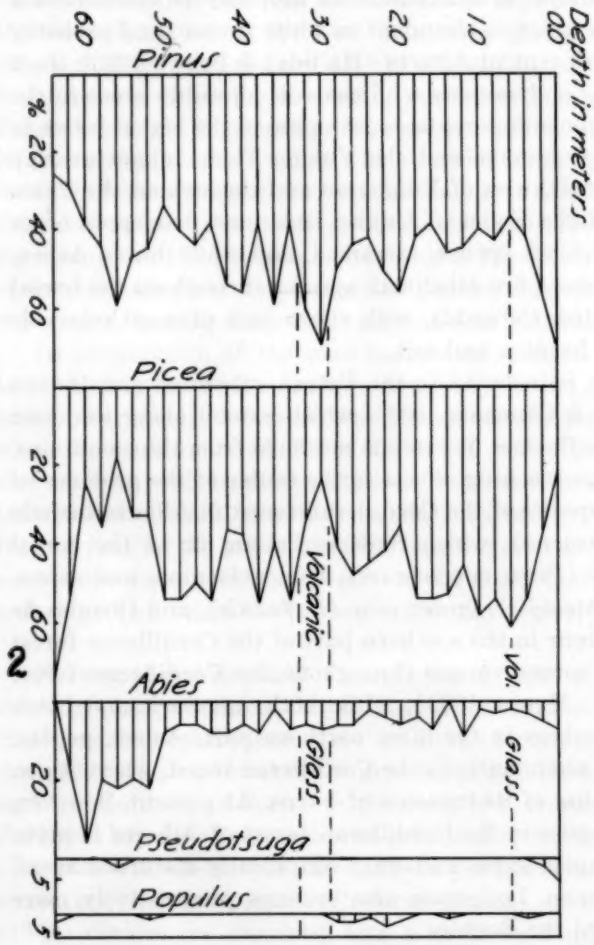

FIG. 1. Pollen diagram of the Duffield bog.

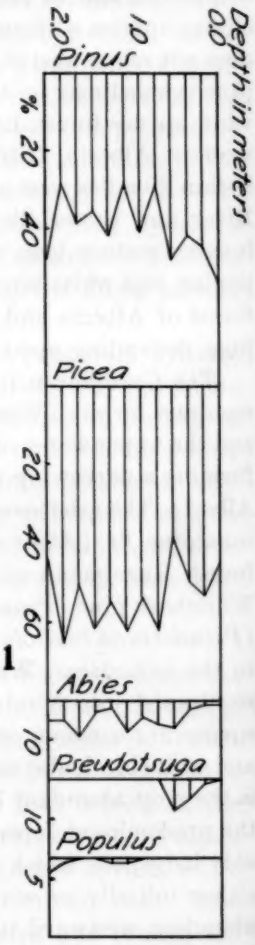

FIG. 2. Pollen diagram of the Entwhistle bog.

white spruce of lodgepole burns suggests that its postglacial record in conjunction with that of spruce and pine would reflect periodic pyric influence more than climatic trends. The interpretation of the pollen profiles largely upon the basis of competition between pine and spruce may not present a true picture of the relative influence of climate and fire on adjacent forest

successions. Practically all of the pine pollen was identified as lodgepole, and interpretation of the profiles is based upon this species. Both black and white spruce pollen was separated in significant proportions, but their individual profiles apparently do not present a true picture of their relative importance in upland forest and bog succession. Black spruce pollen should logically increase in proportion in the upper levels of the peat sections because of its invasion of the mature bog, but there is no consistent pattern of relative proportions of black and white spruce in this respect. Incompetency of the size range method in separating these two species may be responsible for this anomaly. In general, however, white spruce pollen is more abundant than that of black spruce throughout the sedimentary columns, suggesting that the presence of white spruce in adjacent upland forests had a greater influence upon the relative amounts of the two species of pollen in the peat.

Interpretation of the pollen profiles must be based largely upon pine and spruce fluctuations, although some fir pollen is present and its profiles provide a definite trend. Fluctuations of spruce and pine have been largely opposite to each other throughout, indicating their reciprocal successional relationships, perhaps in response to fire and edaphic disturbances, or on mature bogs (figs. 1, 2, 3, 4). The many sharp, opposite fluctuations from level to level fail to present a trend denoting that some consistent, regional influence was at play. Also, the lack of correlative over-all trends in the four sections does not provide either regional patterns or a progression of some influence in an east-west axis that might have been caused by increase in elevation westward or the relative influences of the Keewatin ice advancing from the east and the Cordilleran glacier moving outward on the east slope of the Rockies.

In the Duffield section, spruce has been largely predominant throughout, with pine gaining a slight advantage near the top, which may reflect the influence of fire since the advent of white man (fig. 1).

In the Entwhistle section, which is the deepest, spruce is predominantly recorded at the bottom with 74 per cent, which is its maximum of all profiles (fig. 2). Throughout the section, however, pine and spruce fluctuate conversely to each other, which may be attributed to local periodic fires, disease, or variables inherent in the method of pollen analysis. The only suggestive trend is the maximum of pine attained at 3 m., the same level at which volcanic glass occurs, which may represent the influence of the climatic maximum between 8,000 and 4,000 years ago. A decline of pine above this level may reflect an increase in moisture, while a sharp increase of pine at the top may record the effects of burning augmented by white man.

In only the McKay section is lodgepole predominantly recorded at the bottom in significant proportions (fig. 3). It remains predominant throughout, which may reflect the influence of large adjacent sandy areas on which lodgepole forms dense, pure stands.

The Edson section, which is alomst as thick as the Entwhistle column, perhaps shows more definite trends than the others. Pine and spruce are about equal at the bottom, but spruce increases and pine declines in the next

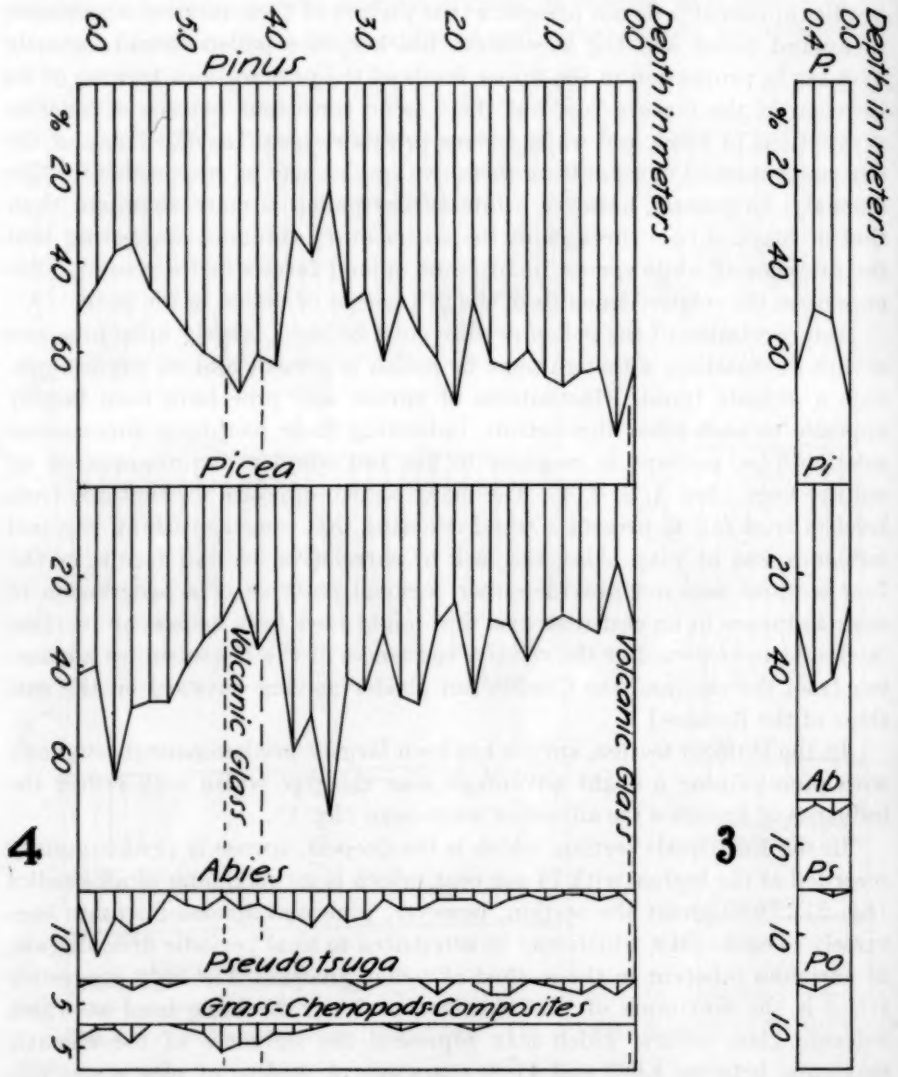

FIG. 3. Pollen diagram of the McKay bog. P, *Pinus contorta*; Ab, *Abies*; Pi, *Picea*; Ps, *Pseudotsuga taxifolia*, PO, aspen. FIG. 4. Pollen diagram of the Edson bog, showing volcanic glass layer through grass maximum.

few levels (fig. 4). They reverse their trends with pine rapidly expanding to 69 per cent and spruce diminishing to 23 per cent at 4.4 m., at which horizon

volcanic glass was noted. If this is chronologically correlated with the 3 m. level in the Entwhistle sections where glass also occurs, this increment in pine may reflect a decrease in moisture. A third sharp reversal of trends reveals a decline of pine to 24 per cent at 3.4 m., its lowest point of the profile, and an increase in spruce to 73 per cent, its highest proportion. From these horizons, pine gradually expands and spruce reciprocally declines toward the top, where they are recorded as 64 and 32 per cent respectively, again perhaps representing the influence of fire and the general over-all more favorable conditions for lodgepole pine at higher elevations toward the Rocky Mountains. If the Edson and Entwhistle sections are chronologically correlated by their respective volcanic glass levels at 4.4 and 3.0 m. respectively, either the Edson column is younger than the other or the rate of peat deposition has been considerably slower in the former, as the ratio of thickness below the glass is almost 1:2. It has been suggested that the Edson section is younger than those farther east, perhaps because of its proximity to the Cordilleran glacier and the resultant physiographic instability during glacial retreat, accentuated by the site of the Edson bog in a valley. On the other hand, it implies a more rapid rate of peat deposition above the glass horizon in the Edson bog, which might be attributable to moister climate nearer the mountains.

The profiles of fir, representing largely subalpine fir, show a consistent trend which may be of some climatic significance (figs. 1, 2, 3, 4). In the Edson and Entwhistle sections, fir is best represented near the bottom, and attains its maxima a few levels higher. From these maxima, fir declines in both sections to proportions less than 10 per cent at the top. As the present range of subalpine fir indicates that it likes a cold climate, its greatest representation near the bottom suggests a cooler climate at that time than now exists. Its higher proportions in the Entwhistle section throughout, however, is inconsistent with the present range of fir. It becomes more abundant westward at higher elevations in the Rockies and one would logically expect it to be better represented in the Edson section, which is nearer the mountains. The higher proportions of fir in the lower horizons of the Entwhistle section suggest that the Edson section is younger. Fir may have existed in greater abundance farther east in ice-free areas during the Late Wisconsin, before the more western Edson bog had initiated pollen-bearing sedimentation. By this time, conditions had become more favorable for spruce, with which fir could not compete.

The occurrence of ice-free areas west of the Altamont border during the Late Wisconsin, as indicated by forest composition, is further supported by the pollen profiles of several bogs east and south of Edmonton. In six of seven sections, pine is predominant when pollen-bearing deposition began (Hansen 1949). These bogs lie east of the lodgepole pine range and most of

the pine pollen in the lower levels was identified as jack pine. This record is quite different from that in the Great Lakes region and eastern North America, where spruce is consistently recorded as the predominant, pioneer, postglacial invader of deglaciated moraine, with the exception of northwestern Wisconsin, where jack pine was apparently co-abundant with spruce on sandy soils (Wilson 1938). These spruce forests with some fir, were replaced by pine as the climate became warmer and drier. This anomaly might be explained by one or more of the following causes and conditions. First, the pollen record does not include the earliest post-Altamont forests which may have consisted largely of spruce and fir, owing to a more rigorous climate. Second, spruce forests may have existed in adjacent ice-free areas before and during the Altamont stage, but conditions favored pine expansion so that it was still predominant when the earliest pollen-bearing sediments were deposited. Third, if the pollen profiles are entirely post-Altamont, the edaphic conditions may have been more favorable for pine than spruce because of physiographic instability resulting from the nearby glacier. Fourth, the early post-Altamont climate may have been milder and drier here than in the Great Lakes region and farther east. Fifth, strong prevailing westerly winds may have carried pine pollen from the Cordilleran forest, where conditions for pine expansion and persistence had been favorable longer.

In this study, however, a somewhat different analogy must be employed, because most of the pine pollen apparently is that of lodgepole pine. In the Pacific Northwest, pollen analysis of many peat sections reveals that lodgepole was consistently the predominant, pioneer, postglacial invader of deglaciated terrain (Hansen 1947). While the ecologic requirements of lodgepole and jack pine seem to be somewhat similar, their relationships to the climax species in their respective areas seem to be slightly different. Both thrive as a result of fire and edaphic disturbance, but lodgepole seems to have a greater ecologic amplitude and is better able to replace the climax dominants. Its range also suggests that it can stand a more rigorous environment than jack pine, while its invasion of mature bogs suggests it has a wider range of soil tolerance. Because of these characteristics, lodgepole is not so good a climatic indicator as jack pine with respect to their respective climax dominants. In the Pacific Northwest, it seems probable that early postglacial physiographic and edaphic instability was favorable for lodgepole in the absence of competition by Douglas fir and western hemlock (*Tsuga heterophylla*). In western Alberta, however, the postglacial co-abundance of pine and spruce, the latter a climax dominant, may represent edaphic and pyric influence more than climatic. Both the sandy or gravelly soil and frequent fires have been favorable for the persistence of lodgepole in abundance. It should be mentioned, however, that with species of wide geographic range,

ecologic races with different ecologic amplitudes may well exist. The abundance of lodgepole pine in west central Alberta peat sections may not mean the same as its early postglacial predominance in the Puget Sound region. Its predominance on sterile pumice mantles in the Oregon Cascades would seem to be more nearly analogous to its co-abundance with spruce in west central Alberta.

The abundance of lodgepole pine when the earliest pollen-bearing sediments were deposited tends to support Hultén's theory that arctic and boreal floras had their greatest spread from centra in Beringia south and east into North America largely by the time of the Yarmouth interglacial (Hultén 1937). They survived the Illinoian and Wisconsin glacial stages in ice-free areas either at the perimeter of the glacier or possibly within the glaciated region. As the glaciers receded, the plants invaded deglaciated terrain from these refugia. Lodgepole pine is considered to be one of a group of continental western American radiants that had migrated south of the 40 parallel and had re-established itself northward by the time of the Late Wisconsin. During this last glacial stage it may have survived in the ice-free corridor between the Cordilleran and Keewatin glaciers. That it also survived in abundance immediately south of the Late Wisconsin ice border in the Puget Sound region and eastern Washington is well evidenced by its preponderant pollen representation at the bottom of many peat sections from these areas (Hansen 1947). Subalpine fir is also classified as a continental western radiant species and its appreciable proportions in the lower levels of all sections further supports its pre-Late-Wisconsin persistence on the east slope of the Rockies in west central Alberta. Neither of these species has spread very far east, and their interglacial and postglacial migrations have apparently been north-and-south and confined largely to the Rockies and the Coast Ranges. Raup (1947) would include *Picea glauca* and *P. mariana* with Hultén's continental western American radiants, but it seems probable that they found refuge south of the ice largely east of the Rockies and spread both east and west during pre-Late-Wisconsin interglacial stages. During at least the latter part of the Late Wisconsin glaciation, lodgepole and spruce occupied the ice-free area on the east slope of the Rockies in west central Alberta, and fire and edaphic disturbance occurred at sufficiently close intervals to favor the persistence of a high proportion of lodgepole in the forest complex.

Other conifers represented sporadically and sparsely in the peat sections include Douglas fir and tamarack. The latter is poorly represented considering its abundance on the bog surface. Douglas fir pollen probably drifted considerable distances, largely from the Rockies, where it occurs as far north as Jasper. It is most consistently recorded in the Edson section, which is to be expected because of its proximity to the mountains (fig. 4). *Populus* is

also sporadically recorded, but in insufficient proportions to be of any interpretive significance except that the pollen of this species is poorly preserved in peat. Fairly high proportions of *Populus* pollen were noted in a few bogs about Edmonton (Hansen 1949). Pollen identified as that of poplar occurs in all but the Edson bog, and this is also consistent with the composition of adjacent forests, as lodgepole becomes more abundant farther west at the expense of aspen. In the Edson section, the author was surprised to note grass, chenopod, and composite pollen in many levels, with grass the most common (fig. 4). It may be merely coincidental, but the volcanic glass which has been tentatively assigned to Glacier Peak which apparently erupted near the time of the postglacial climatic maximum as indicated by many pollen profiles in Washington, occurs at the same levels as the grass maximum. As the climatic maximum in eastern Washington is portrayed by a sharp influx of these drouth indicators, it may be possible that the influence of this warm, dry interval was felt in west central Alberta. A significant maximum of this group of xeric indicators also occurs in the peat sections east and south of Edmonton, which further supports this evidence.

SUMMARY

Pollen analysis of four peat sections from bogs lying between Edmonton and the Rockies in west central Alberta, reveal that lodgepole pine and spruce have been the predominant forest tree species in adjacent areas during the time represented by the sedimentary columns. Since these bogs lie west of the border of the Altamont (Late Wisconsin) moraine and on older Keewatin drift, but probably east of the Late Wisconsin Cordilleran ice border, it is possible that they record pre-Late-Wisconsin forests, that had persisted in an ice-free corridor during this glacial stage. This is consistent with Hultén's theory that continental western American radiants from a Beringia center had migrated southward before the Wisconsin glaciations and persisted ice-free areas close to or within the ice front during at least the Late Wisconsin stage. The postglacial warm, dry interval, evidenced from so many sources, may be slightly recorded by an influx of grasses with some chenopods and composites in the vicinity of the Edson bog, 125 miles west of Edmonton.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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NOTE ON THE SECTION MICROPHYLLAE OF AGROSTIS

ALAN A. BEETLE

At the time of the original description of the section *Microphyllae* Beetle of *Agrostis* (Bull. Torrey Club **72**: 541–549. 1945) all known species with awned glumes were treated. It was found that two other characters, a bent, exserted awn and absence of the palea, were correlated with the awning of the glumes. Since 1945 two new species have been described which would fall into the section *Microphyllae* of *Agrostis* with the exception that both have well developed paleae.

Agrostis aristiglumis Swallen (Leaf. West. Bot. **5**: 56. 1947), which falls well within the geographic range of the four species originally listed, is unquestionably a member of the section, and requires the emending of the original concept of the section to include both the presence and absence of a palea.

Deyeuxia parsana Bor (Kew Bull. **1948**: 42. 1948) also described with awned glumes—"ab omnibus speciebus adhuc descriptis glumis aristatis facile distinguitur"—undoubtedly belongs in the same section. It is a perennial described from "Persia. Shah Zadeh Kuh, 3100 m., coll. Dr. A. Parsa Typus in Herb. Kew." and extends the range of the section into the Old World for the first time. Like *Agrostis aristiglumis* Swallen this species has an exserted awn of the lemma and a well developed palea. In order to place it properly in a section of *Agrostis* the following transfer is made: **Agrostis parsana** (Bor) Beetle, comb. nov.

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NEW SPECIES OF CYPERACEAE FROM BRITISH GUIANA AND SURINAM

CHARLES L. GILLY

From among the specimens of northern South American Cyperaceae examined during the past year,[1] the following three species merit recognition.

TRIBE HYPOLYTREAE

Hypolytrum sandwithii Gilly, sp. nov. Herba perennis, caespitosa?; caulis brevis lignosus; folia lineari-elliptica glabra (concolora?), 5–6 dm. longa and 2.3 cm. lata, margine minute serrulata, basi in petiolum canaliculatum attenuato, ad apicem longe acuminata, acumine ad 1 dm. longo; culmi floriferi solitarii in axilibus foliorum, subtriangulares glabri vel minute pulverulentes et supra ad angulos minute serrulati, cum inflorescentia 2–3 dm. alti; inflorescentia subcorymbosa, spiculae glabrae in glomerulis dispositae; glomeruli 0.8–1.2 cm. diam., solitarii ad apices ramulorum ad 2 cm. longorum, glomerulo ultimo sessili; bracteae inferiores inflorescentiam subtendentes foliis consimiles, ad 2.5 dm. longae; bracteae superiores glomerulos subtendentes lanceolatae acuminatae ad 1.5 cm. longae; flores in spiculis bisexualibus monoecis; spiculae bracteolis hyalino-brunneis lanceolatis vel oblongo-lanceolatis acutis vel obtusis ad 2.5 mm. longis subtentae; glumae hyalino-brunneae glabrae; glumae vacuae exteriores 2, subcarinatae lanceolatae vel elliptico-lanceolatae, 3–3.5 mm. longae; glumae flores masculos subtendentes planae lineari-lanceolatae vel anguste lanceolatae, ad 2.5 mm. longae; flores masculi 4–6 monandri in parte spiculae inferiori, perianthio nullo, antheris ad 1 mm. longis, filamentis persistentibus; flores feminei in parte spiculae superiori et media, perianthio nullo; achaenium subteres vel subtereti-lenticulare subulato-conicum, ad 1 mm. latum et cum rostro spongioso glabro et persistens 4–5 mm. longum; corpus achaenii stramineo-olivaceum, cum rostro minute et dense purpureo-maculatum; stylus bifidus, stigmata gracilia atro-brunnea. BRITISH GUIANA: Potaro River, Kaieteur Falls: by stream in high forest, descending from savanna to falls, "growing with spp. of Rapateaceae . . . only this specimen collected; no others seen with inflorescence," altitude ca. 400 m., September 5, 1937, *N. Y. Sandwith 1379* (K—TYPE; NY—drawing and fragment; ISC—photograph and fragment).

[1] Specimens reported upon in this paper may be found in the herbarium of the Royal Botanic Gardens, Kew, England (K) or in the herbarium of the New York Botanical Garden (NY), and duplicates or photographs and accompanying fragments are in the herbarium of Iowa State College (ISC); isotypes of the two members of the Cryptangieae presumably have been distributed to still other herbaria. I wish to express my thanks to the curators of these herbaria for permission to examine the specimens, and to offer particular thanks to Mr. N. Y. Sandwith for his courtesy in referring the specimen of *Hypolytum* to me for study and for his patience in awaiting determination.

In the structure of the achene beak, this species apparently is related to *H. strictum* Kunth (see Clarke 1909, *pl. 103, f. 18*), and in the type and arrangement of leaves it is very similar to *H. Jenmani* C. B. Clarke; however, *H. sandwithii* differs markedly from both of these species in the details of inflorescence, spikelets and achenes. The long-acuminate leaf apices are distinctive in the genus. It is of interest to note that *H. sandwithii* greatly resembles some of the species of the related genus *Bisboeckelera* Kuntze, particularly *B. irrigua* (Nees) Kuntze of northwestern Brazil and *B. longifolia* (Rudge) Kuntze of Surinam and French Guiana.[2]

TRIBE CRYPTANGIEAE

Cephalocarpus maguirei Gilly, sp. nov. Caulis multiramosus ad 1.5 dm. altus, foliis persistentibus tectus; folia linearia acuta bicarinata glabra margine costaque albo-pilosulis et carinis uncinato-papillosis exceptis, ad 5 cm. longa et 2 mm. lata; culmi floriferi solitarii in axilibus foliorum, 4.5–6 cm. alti; bracteae inflorescentiam subtendents ciliatae and 8 mm. longae; spiculae masculae 3.5–4 mm. longae, glumis vacius 3–5 lanceolatis acutis vel mucronulatis glabris, glumis staminiferis 2–3 lanceolatis acutis glabris; antherae deciduae ignotae; spiculae femineae 3.5–4 mm. longae. glumis 5–8 ovatis vel lanceolato-ovatis mucronatis glabris; achaenia subteretia glabra atrobrunnea ad 1.5 mm. longa et 1 mm. diam., rostro 0.5–0.7 mm. longo luteo glabro deciduo, apiculo 0.1–0.2 mm. longo; squamellae hypogynae minute orbiculares, ciliis paucis longitudine partem tertiam corporis achaenii aequantes. BRITISH GUIANA: Kaieteur savannas, "forming tussocks . . . rare. . . ." May 14, 1944, *B. Maguire and D. B. Fanshawe 23453* (NY—TYPE).

A member of the subgenus *Neocephalocarpus* by virtue of the solitary flowering culm in each leaf axil. Characters of the achene and the deciduous beak, however, have been observed previously only in the subgenus *Eucephalocarpus;* an eventual realignment of the intrageneri subdivisions of *Cephalocarpus* (Gilly 1942) may be in order. This specimen has been previously recorded (Svenson 1948) in the literature as "*Cephalocarpus* aff. *rigidus* Gilly."

Everardia surinamensis Gilly, sp. nov. Caulis gracilis, ad 8 cm. altus, vaginis persistentibus brunneis fimbriatis tecta; folia plana glabra margine costaque albo-pilosulis exceptis vel subtus sparse pilosula, ad 3.5 dm. longa et ad basim 1 cm. lata; culmi floriferi minute pubescentes laxe adscendentes

[2] Although superficially resembling and first placed in the Cariceae, *Bisboeckelera* has been assigned by various authors to the Cryptangieae, the Sclerieae or to the Bisboeckelerieae. Its affinities, however, are with *Hypolytrum, Mapania,* and related genera of the Tribe Hypolytreae; the utriole ("false perigynium" of some authors) of *Bisboeckelera* apparently has been developed through embryonic coalescence, or failure of embryonic separation, of sterile glumes such as occur between the staminate flowers and the pistillate flower in *Lepironia, Mapania,* and *Thoracostachyum.* The relationships in this complex will be considered in detail at a later date. The genus *Bisboeckelera* has been discussed at length by both Standley (1914) and Pfeiffer (1937).

vel erecti, cum inflorescentia ad 4 dm. alti; vagina ad basim 3 cm. longa; vaginae floriferae brunneae minute pubescentiae laminas subaequantes; spiculae masculae numerosae 3–4 mm. longae, glumis vacuis 3–6 ovatis vel ovato-lanceolatis acutis vel mucronatis glabris, glumis staminiferis 3–5 lanceolatis vel oblongo-lanceolatis acutis vel mucronulatis glabris; spiculae femineae 2.8–3.2 mm. longae, glumis 5–6 ovatis vel ovato-triangularibus mucronatis; achaenia brunnea anguste ellipsoidea, ad apicem sparse et minute pubescentia, cum rostro 2.5–2.8 mm. longa, 0.5 mm. diam.; rostro conico pubescenti, quam corpore achaenii dimidio brevioribus; squamellae hypogynae minutae suborbiculares, ciliis paucis quam corpore achaenii brevioribus. SURINAM: Tafelberg (Table Mountain): wet places in dense low makka swamp, near Savanna no. IV, altitude 520 m., August 15, 1944, *B. Maguire 24382* (NY—TYPE); moist mossy rocks, base of wall, West Escarpment, altitude 485 m., September 10, 1944, *B. Maguire 24679* (NY).

Vegetatively similar to *E. montana* Ridley (see Gilly 1940) from Mount Roraima and Ptari-tepui in Venezuela, as previously indicated by Svenson (1948), but differing greatly from that species in structure and pubescence of the achene as well as in minor vegetative characteristics.

It is also of interest to note that a staminate specimen of the genus *Didymiandrum* [BRITISH GUIANA: savannas on the Kaieteur plateau, May 5, 1944, *B. Maguire & D. B. Fanshawe 23230* (NY)]—appears to be more nearly referable to *D. guaiquinimae* Schnee (Schnee 1943) than to *D. stellatum* (Böckl.) Gilly, as recently reported by Svenson (1948). *D. guaiquinimae*, originally described from pistillate plants collected on Cerro Guaiquinima in southeastern Venezuelia, has been collected more recently by Steyermark on Ptari-tepui, another cerro in the same area.

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NOWAKOWSKIELLA CRASSA SP. NOV., CLADOCHYTRIUM AUREUM SP. NOV., AND OTHER POLYCENTRIC CHYTRIDS FROM MARYLAND[1]

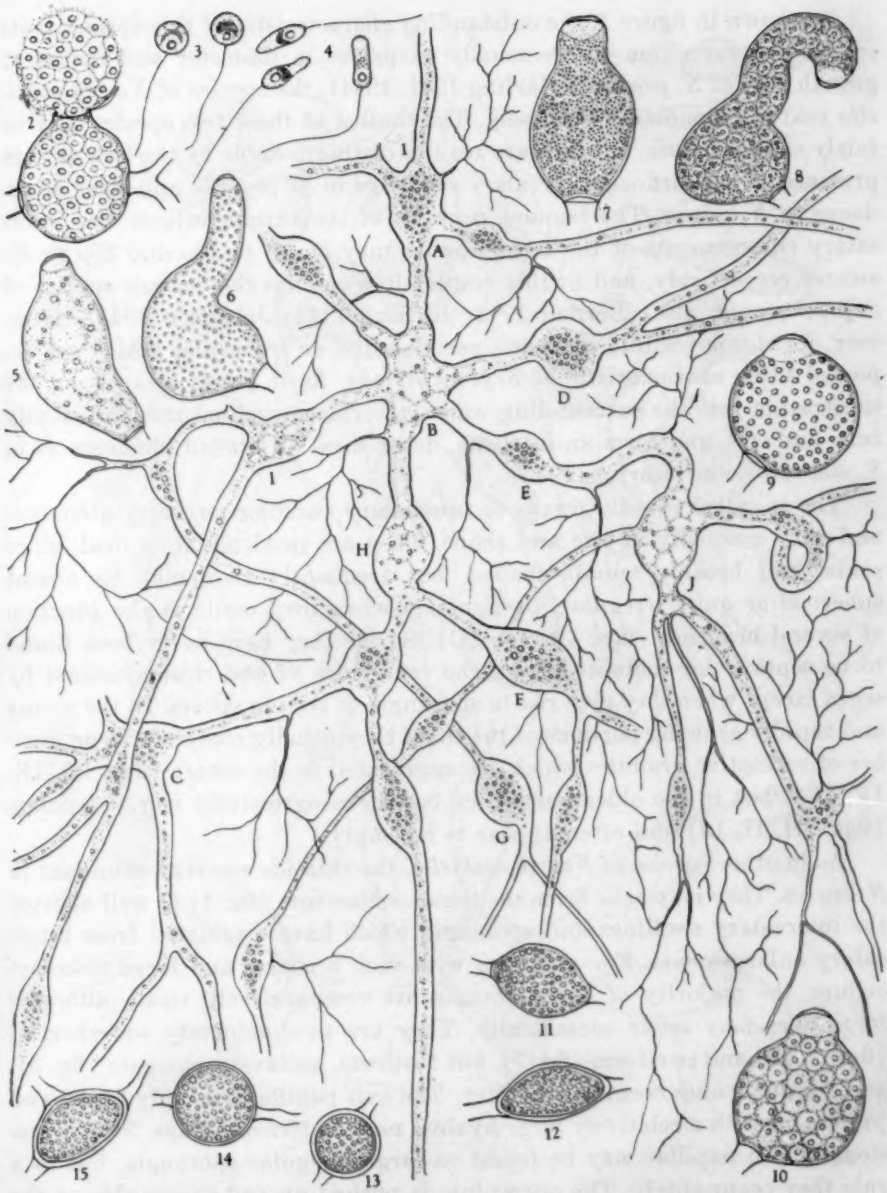
JOHN S. KARLING

In the course of a survey of the soil fungi of Maryland a number of polycentric chytrids were isolated on cellulosic, chitinic, and keratinic substrata, and among these were two new species of the family Cladochytriaceae. Both of these chytrids were isolated from a mixture of soil and water and grown on floating bits of onion scale. It was found later, however, that they may grow to some extent on dead human skin also, but not on chitin. The first of these species is characterized by an usually large and coarse rhizomycelium without pseudosepta or trabeculae, nonseptate intercalary swellings, and operculate sporangia, and belongs in the genus *Nowakowskiella*. It differs fundamentally from the other species of this genus by its exceptionally large thallus, and for this reason it is diagnosed as a new species and named *N. crassa*. The second species is distinguished by a very delicate rhizomycelium, septate intercalary enlargements, and inoperculate sporangia. Accordingly, it belongs in the genus *Cladochytrium*, and is strikingly similar to *C. tenue* in most respects. However, it differs from this species by its larger zoospores and the presence of a highly refringent golden-orange to red globule in the latter. In this respect its zoospores resemble those of *C. replicatum*, but otherwise the two species appear to be different and distinct. The Maryland species is, therefore, interpreted as a new species for which the name *C. aureum* is proposed.

Nowakowskiella crassa Karling, sp. nov. Fungus saprophyticus. Rhizomycelio hyalino, profuso, ramoso, partibus tenuibus 6–18μ diam., incrementis pluribus non septatis, subsphaericis, 14–21μ, ovalibus, 12–18 × 16–22μ, fusiformibus, 14–16 × 25–28μ, aut irregularibus. Sporangiis terminalibus aut intercalaribus, hyalinis, levibus, sphaericis, 15–40μ, ovalibus, 18–30 × 22–38μ, fusiformibus, clavatis, aut irregularibus; operculo 10–15μ diam. Zoosporis sphaericis, 4.5–5μ diam. unico globulo refringenti, 0.8–1.2μ diam; flagello 24–26μ longo. Sporis perdurantibus hyalinis, levibus, sphaericis, 12–23μ, ovalibus, 18–20 × 22–24μ; germinatione ignota.

Saprophytic in dead and disintegrating vegetable debris, small brook on route 506 near Prince Frederick, Calvert County, Maryland.

[1] This work was begun at the Chesapeake Biological Laboratory, Solomons, Md., where the writer was guest investigator during the spring and early summer of 1948. The author is deeply indebted to the Department of Education and Research, State of Maryland, and particularly to the Director of the Laboratory, Dr. R. V. Truitt, for providing research facilities and funds.



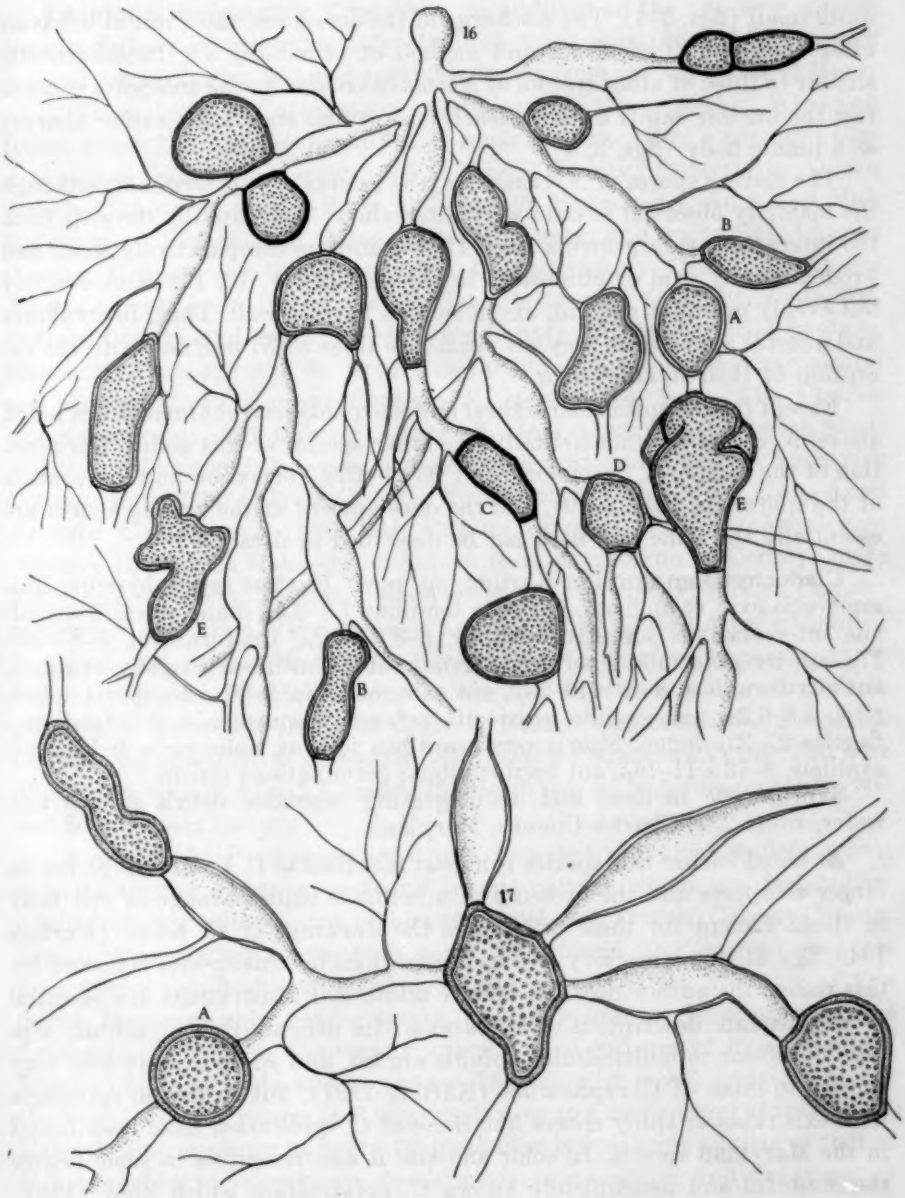
FIGS. 1–15. *Nowakowskiella crassa.* FIG. 1. Portion of vegetative rhizomycelium showing variations in size and shape of intercalary swellings and habit of branching, ×800. FIG. 2. Dehiscence of sporangium, ×800. FIG. 3. Zoospores in motile phase, ×1450. FIG. 4. Creeping, amoeboid zoospores, ×1450. FIGS. 5–10. Successive maturation stages in fully-formed and variously-shaped sporangia, ×800. FIG. 11. Early developmental stage of intercalary resting spore, ×800. FIGS. 12–15. Variations in size and shape of resting spores, ×800.

As shown in figure 1, the outstanding characteristic of this species is its coarse rhizomycelium which usually surpasses in diameter and extent of growth that of *N. profusa* (Karling 1941, 1944), the species of *Nowakowskiella* that it resembles most closely. The thallus of these two species may be fairly similar in size, but they are readily distinguishable by the lack or rare presence of well-defined intercalary swellings in *N. profusa* and their abundance in *N. crassa*. The tenuous portions of the rhizomycelium and intercalary enlargements of the latter species may be up to 18μ and 25μ in diameter respectively, and in this respect it resembles the coarser species of *Septochytrium* also (Berdan 1942; Karling 1942; Johanson 1943). However, its rhizomycelium lacks the pseudo-septa or trabeculae which are reported to be characteristic of *Septochytrium*. As it grows away from the substratum into the surrounding water, the rhizomycelium may frequently turn and coil, and form an intricate, dense mass of twisted filaments as in *S. macrosporum* (Karling 1945).

The so-called spindle organs or intercalary swellings are very abundant and vary markedly in size and shape. They are predominately oval, ellipsoidal and broadly spindle-shaped, but frequently they may be almost spherical or quite irregular, particularly when they occur at the junction of several branches (figs. 1A, 1B, 1C). So far they have never been found to be septate nor delimited from the remainder of the rhizomycelium by septa except when they give rise to sporangia or resting spores. In the young and rapidly growing portions of the thalli they usually contain a large number of refractive granules which are aggregated in the center (figs. 1D, 1E, 1F, 1G), but in the older parts they become progressively more vacuolate (figs. 1H, 1I, 1J) and often appear to be empty.

As in other species of *Nowakowskiella*, the rhizoids are very abundant in *N. crassa*. They may arise from the tenuous filaments (fig. 1) as well as from the intercalary swellings and sporangia which have originated from intercalary enlargements. For a species with such a coarse and large rhizomycelium, the majority of the sporangia are comparatively small, although large ones may occur occasionally. They are predominately subspherical (figs. 2, 10) and pyriform (fig. 7), but fusiform, obclavate, elongate (fig. 5), and irregular ones occur fairly often. The exit papilla is usually broad and prominent with a relatively large hyaline zone underneath (figs. 5–9). Occasionally two papillae may be found on large irregular sporangia, but as a rule they occur singly. The operculum is pushed up and to one side as the zoospores emerge (fig. 2) and remains attached for several hours after dehiscence.

The zoospores of this species are comparatively small, and as in *N. profusa* and *N. delica* (Whiffen 1943) their hyaline refractive globule is



FIGS. 16, 17. *Polychytrium aggregatum* (?). FIG. 16. Portion of a thallus on chitin showing the abundance, size, shape, content and color of the resting spores, × 800. FIG. 17. Enlarged portion of the thallus showing thickening of the walls of the intercalary enlargements, × 1100.

quite small (figs. 3–4). The discharge of the zoospores, their initial behavior after emerging (figs. 3, 4), and method of swimming are fundamentally similar to those of other species of *Nowakowskiella*. As the zoospores come to rest the nuclear cap is usually clearly visible and stands out rather sharply as a lunate body (figs. 3, 4).

The resting spores of *N. crassa* may be intercalary or terminal, although the majority observed to date were intercalary. They usually develop from the intercalary enlargements (figs. 11–15) and are comparatively small and predominantly oval or ellipsoidal in shape (figs. 11, 12, 15) with coarsely but evenly granular content, and a smooth hyaline wall. Thus, in structure and general appearance they are similar to those of *N. profusa*, with the exception of their hyaline color.

Except for the specific structural and morphological characteristics noted above, *N. crassa* is fundamentally like other species of this genus. Germination of the zoospores, development of the thallus, formation and maturation of the sporangia (figs. 5–10), and the development of the resting spores are essentially the same and need not be described in detail.

Cladochytrium aureum Karling, sp. nov. Fungus saprophyticus. Rhizomycelio exili et angusto, partibus tenuibus 1.2–3.5μ diam., amplificationibus intercalaribus septatis, ovalibus, 6–8 × 9–12μ, fusiformibus, 5–8 × 10–17μ, aut irregularibus. Sporangiis terminalibus aut intercalaribus, ovalibus, aut citriformibus, 8–19 × 10–23μ, aut subsphaericis, 8–36μ. Zoosporis sphaericis, 5.8–6.2μ, uno globulo luteo-rufo refractivo conspicuco, 2–2.4μ diam.; flagello 27–29μ longo. Sporis perdurantibus levibus, sphaericis, 9–17μ, aut ovalibus, 8–12 × 11–16μ, aut fusiformibus; germinatione ignota.

Saprophytic in dead and disintegrating vegetable debris in soil and water, route 234, Charles County, Maryland.

As noted earlier this species is almost identical to *C. tenue* except for its larger zoospores and the presence of a brilliant golden-orange or red body in them. Except for these differences the drawings of *C. tenue* (Karling 1945, figs. 31–45) serve very well as illustrations for this species also, and for this reason the author does not believe additional illustrations are essential to an adequate description of *C. aureum*. Its delicate rhizomycelium, septate, bicellular to multicellular spindle organs, and zoospores are also very similar to those of *C. replicatum* (Karling 1931), but so far no sporangia with exit tubes or spiny spores like those of *C. replicatum* have been found in the Maryland species. In color and size it also resembles to some degree the doubtful and incompletely known *C. polystomum* which Zopf (1884) illustrated but did not describe. However, in this species the sporangia bear several long exit tubes, which apparently are lacking in *C. aureum*. The other fully known species of *Cladochytrium*, *C. crassum* (Hillegas 1941) and *C. hyalinum* (Berdan 1941) are hyaline, markedly larger, and quite distinct from the present species.

Additional Polycentric Chytrids. In addition to the two new species described above several other polycentric chytrids were isolated from water and soil containing animal and vegetable debris from various parts of Maryland. These include *Catenomyces persicinus* (Hanson 1945), Wicomico River, route 234, Charles County and route 236, St. Mary's County; *Cladochytrium replicatum*, route 505, Calvert County, Centerville, Queen Anne's County, Monocacy River at Route 40, Frederick County and Fish Hatchery on Severn Run, Anne Arundel County, and route 234, Charles County; *C. hyalinum*, Old Spout Farm, Calvert County, Centerville, Queen Anne's County, and Savage River, Garrett County; *C. tenue*, route 504, Calvert County and route 234, Charles County; *Nowakowskiella profusa*, Monocacy River at route 40, Frederick County, route 234, Charles County, and Savage River, Garrett County; *N. macrospora*, route 234, Charles County and route 505, Calvert County; *N. elegans*, route 300 between Sluderville and Church Hill, Queen Anne's County, Stonsifer's farm, Frederick County, route 509, Calvert County, Fish Hatchery on Severn Run, Anne Arundel County, and route 234, Charles County; *N. hemisphaerospora* (Shanor 1942) Fish Hatchery on Severn Run, Anne Arundel County, route 234, Charles County, Monocacy River at Miller's Bridge, Frederick County, routes 509 and 505, Calvert County, and route 300, Queen Anne's County; *N. granulata*, route 504, Calvert County; *Septochytrium variabile*, route 503 to Drum point Beach, Calvert County; *Myceliochytrium fulgens* (Johanson 1945), Monocacy River at Miller's Bridge and Keesville, Frederick County, Wicomico River and route 234, Charles County, and route 300 near Sluderville, Queen Anne's County; *Polychytrium aggregatum* on routes 503 and 504, Calvert County.

In relation to *P. aggregatum* it may be noted that thick-walled, brown cysts or resting spores were found in one culture which was isolated on chitin from wet soil collected besides route 504 near Dowell, Calvert County. Inasmuch as resting spores have never been found in *Polychytrium*, this discovery is significant in extending our knowledge of the life cycle of this genus. However, only resting spores, and no sporangia or zoospores were formed in this culture. Conversely, other typical cultures of *P. aggregatum* formed only sporangia and zoospores. Therefore, it is not certain whether the Dowell culture relates to *P. aggregatum* or to a new species of this genus. Nevertheless, as shown in figure 16, its thallus is strikingly similar to that of *P. aggregatum* in appearance, development, organization, and structure.

The resting spores begin as hyaline, relatively thin-walled, enlargements or lateral outgrowths of the rhizomycelium in which the dense, finely granular protoplasm accumulates. As they increase in size they are delimited from the remainder of the thallus by cross septa and gradually become thick-walled and brown in color. It is not certain, however, that this color is a

specific characteristic because the whole rhizomycelium may take on the color of the surrounding water and turn brown with age. The intercalarly enlargements and tenuous portions of the rhizomycelium may also become thick-walled, as shown in figure 17. The mature resting spores vary markedly in size and shape, as shown in figure 16. They may be almost spherical (fig. 17A), oval, 10–24 × 14–30μ (fig. 16A), oblong, 12–16 × 22–34μ, elongate, 9–12 × 22–38μ (fig. 16B), truncate (fig. 16C), angular (fig. 16D), deeply lobed (fig. 16E), or very irregular. Although several empty spores have been found, it is not yet known whether they give rise directly to zoospores or function as prosporangia in germination.

No evidence of sexuality has been observed in relation to their formation and development, and in this respect *Polychytrium* appears to be very similar to the other genera of the Cladochytriaceae. However, it is not yet certain that it belongs in this family. The fact that no evidence of sexuality has been found does not preclude the possibility that the thallus on which the resting spores are formed may have arisen from a zygote, or that the resting spores may give rise to gametes in germination as in *Catenaria* (Couch 1945) and other members of the Blastocladiales. Therefore, until these details of its life cycle are known the taxonomic position of *Polychytrium* will remain doubtful.

SUMMARY

In a survey of the soil fungi of Maryland two new polycentric chytrids were collected and isolated on cellulosic substrata. The first of these species is characterized primarily by an unusually large coarse rhizomycelium which lacks pseudo-septa or trabeculae, and by operculate sporangia. Accordingly, it belongs in the genus *Nowakowskiella* and is described as a new species, *N. crassa*. The second species belongs in *Cladochytrium* and is distinguished by a very delicate rhizomycelium, bi- to multiseptate spindle organs, inoperculate sporangia and large zoospores which contain a brilliant, golden-orange or red refractive body. It is very similar to *C. tenue* except for its pigmentation and larger zoospores, and because of these characteristic differences it is diagnosed as a new species and named *C. aureum*.

In addition to these species several other saprophytic polycentric chytrids were isolated from soil and water in various parts of Maryland. These include: *Cladochytrium tenue, C. replicatum, C. hyalinum, Nowakowskiella elegans, N. profusa, N. macrospora, N. hemisphaerospora, N. granulata, Septochytrium variabile, Catenomyces persicinus, Myceliochytrium fulgens,* and *Polychytrium aggregatum*. Resting spores were discovered for the first time in *Polychytrium*.

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TORREYA

Vernacular Names for Roccella. An Etymological Note. During the editing of an article by William F. Leggett on "Lichens as Dye Plants" for the Journal of the New York Botanical Garden, where it appeared in May 1949, my attention was drawn so many times to the various designations for the dye-yielding lichens and to their origin, spelling, and even pronunciation (for who can edit a paper without forming the sound of the words in his mind?), that I began to investigate the vernacular names used in different languages for *Roccella*. This, the generic name for the principal dye lichen, *R. tinctoria*, also is one of the names for the plant in Italian, and a word which in turn provided the surname for a famous Florentine family whose wealth was based on its monopoly in the 13th century of the lichen dye industry.

Roccella tinctoria was apparently known as a source of red and purple dye in the Holy Land many hundred years before the time of Christ. Its use in the eastern Mediterranean was the subject of comment by the early naturalists. In the Middle Ages it became known in Italy; later the lichen was discovered in the Canaries and on the west coast of Africa. It is only in fairly recent years that it has been completely supplanted by chemically made dyes—except for the manufacture of litmus paper, for which it still is used.

In English this lichen, and also the dye that it gives, are both generally called "orchil" or "archil."

The origin of the word *orchil* seems to be a matter of conjecture and dispute, even among such authorities as the "Oxford"[1] and "Webster."[2] The spelling and pronunciation of the word are also far from stable. In Webster, *archil* is given as the preferred form, with *orchil, orchal,* and *orchilla* as variants, all with a *k* sound except under the separate entry for *orchil,* where the English *ch* sound is given as second choice.

In the Oxford, on the other hand, the principal entry is *orchil,* given only with the *ch* sound, but with a cross-reference to *archil,* called a corruption of *orchil,* and pronounced with either a *ch* or a *k* sound (the *k* second choice). The date on which both words first occurred in the English language is given as 1483, *orchil* being used by Shakespeare for the dye in Act I of "Richard II"; *archil* referring essentially to the plant in "Richard III."

[1] *The Oxford English Dictionary.* 1933.

[2] *Webster's New International Dictionary of the English Language.* Second edition, unabridged, 1935.

One wonders whether these distinctions, made in a day when the language was in its incipient stages, were actual or unintentional. *Orchil* for the plant was first recorded in 1758 in the Philosophical Transactions of the Royal Society of London. *Orchilla* or *orchella* (with *ch* sound), meaning the same things, did not appear until 1772, according to the Oxford.

It seems curious that a word which was in use in Shakespeare's day should be designated "origin uncertain" in the Oxford and other comprehensive dictionaries.

The Webster unabridged dictionary seems to go far afield when it states, under *archil*, "Perhaps from Latin *herba urceolaris*, a plant used for polishing glass pitchers." But *herba urceolaris* is given in Riddell's Latin-English lexicon as "pellitory-of-the-wall" (*Parietaria officinalis*) of the nettle family (not the common pellitory, which is a small composite resembling chamomile). So far, no reference has been found to suggest that either the flowering plant *Parietaria* or any lichen in the world has ever been used for polishing.

The botanical name of *Roccella* is more closely allied to *orchil* and to the modern French form *orseille*,[3] which is commonly used among dyers in this country today, than at first seems evident. It is a custom of language occasionally to invert an R and a vowel. (It occurred in English in the word "ran," which was originally "arn.") In the Italian vernacular, according to Pietro Antonio Micheli, the great 18th century mycologist, in his "Nova Plantarum Generum" (1729), the lichen used for dyeing was called *roccella*, *orcella*, or *raspa*,[4] and the dye itself was known as *oricella*. The *ch* sound of the Italian *c* when followed by an *e* as in these words, gives good ground for the Oxford preference for the soft *ch* in *orchil*, *orchilla*, and *orchella*—and also gives a logical source for the English name of the plant, however varied it may be. This is a type of word which would enter a language through the speech of workers, rather than through literature. It is therefore the pronunciation rather than the spelling that would be carried over.

The common name of the lichen in Italy goes back long before the date of Micheli, and even before the "Italian Natural History of 1599" mentioned in *A Modern Herbal* by Grieve and Leyel (1931).

An American vice-consul in Florence once asked that a privately printed book which he had seen in Italy be presented to the Astor Library (now part of the New York Public Library). This was a history of the

[3] An earlier French form was *lorchel*, according to Littré, *Dictionnaire de la Langue Française*. *Orseille* is pronounced *or-sale* in both languages, according to Larousse and Littré in French, and Webster in English.

[4] *Respia* and *rusca* are mentioned in other Italian sources, but neither these nor *raspa* seems to have survived.

famous Rucellai family of Florence,[5] and it is one of the few places where the origin of the family name and its connection with the lichen used for dyeing are recounted.

"And our ancestors were lichen dyers," says the author, "and in that period there was no one in Florence nor even in all Italy except these ancestors of ours who knew how to dye with lichens." One of them had gone to the Levant, where the art had been known from the earliest days, and brought the secret of the process back with him. "And this was the cause which gave so much wealth and good living to our family . . . *and from this art of the lichen dye is derived the name of Rucellai*" (italic mine). The name was originally Oricellai (it occurred also in various other spellings), but it is as Rucellai that the family became famous.

The name first achieved prominence about 1250, but it was previous to that time that one Marco di Giunta d'Alamanno di Monte de messer Ferro came to Italy in the retinue of an emperor (possibly Frederick II, grandson of Frederick Barbarossa, who was crowned in Rome in 1220). The stories say he came from Brittany, but from his name one might judge that he had come from Germany (then called Allemania). He settled in Florence and established a successful mercantile business there, dealing in woolens and carrying on commerce with distant places. One of his family, probably Federigo, spent a long time in the islands of the eastern Mediterranean, where he found a native lichen being used as a purple dye. This was obviously the plant which both Theophrastus and Pliny had recorded as growing in rocks along the seashore of Crete, and used for producing a beautiful purple dye on woolens. They both gave the common name of "seaweed" to it, and Pliny noted that no language had any other name for it than that used by the Greeks, *Phycos thalassion*.

So the date when the names of *roccella* and *orcella* came into the language that Dante crystalized is obscured in those first twelve centuries of the Christian era. The Spanish, at some time during that period, adopted the name of *orcigilia*, or *orciglio*, and also used *orchilla* and *orchillo*, and the Portuguese said *urzela* (or *urzella*), all obviously related to the Italian word for the lichen and its dye.

Some of the early botanical authors say that the present generic name for the lichen, *Roccella*, is a diminutive formed from *rocca*, because the species always grows on rocks (botany is full of such "made up" derivations). But this does not seem probable, since the word *rocca* does not appear in the classical Latin from which botanical names are most often derived. It is possible that the Italian vernacular name of *roccella* or *orcella* had this derivation, but the Oxford dictionary dismisses all such

[5] "Un mercante fiorentino, Giovanni Rucellai, e la sua famiglia nel secolo XV" by G. Marcotti, published in Florence in 1881.

claims with the flat statement: "Whether the original was *Oricello* or *Orciglio*, a derivation from *rocca*, rock, founded on modern Latin *roccella*, is out of the question."

Five hundred years after the Rucellai family was established and named for the dye-producing lichen which brought it wealth, Linnaeus, following some of his botanical predecessors, called the plant *Lichen Roccella* in his *Species Plantarum* (ed. 2. 1763). In 1805 Augustin Pyramus de Candolle adopted the name *Roccella tinctoria* for the plant, and this it has remained to the present day.

The name *Lacca musica* also occurs for *Roccella tinctoria* in reference books, but it seems to have no significance, except as a pseudo-Latin form in which *musica* is an oft-repeated error for *musci*. Or, it may be an extension of the two syllables of *lacmus*, which is another name for *litmus*, a coloring substance also derived from this lichen.

These two words pose a similar problem, for if we follow the Webster unabridged dictionary, the *-mus* in *litmus* stems from the Old Norse word for moss, added to *litr*, meaning dye or color. Thus it was recognized early as a dye plant. But the *-mus* in *lacmus* comes from the Dutch *moes*, meaning pulp; the *lac* comes from *lak*, meaning stain, in the original Dutch word.[6] *Lacmus*, therefore, if the difference actually exists, first referred to the dye itself, whereas *litmus*, now seldom thought of except as a changeable coloring matter used for testing acidity and alkalinity, in its original form referred only to the lichen from which the dye was obtained.—CAROL H. WOODWARD.

A Note on Buried Cedar Logs at Secaucus, N. J. In the Hackensack tidal marsh at Secaucus, N. J., a great number of logs of southern white cedar [*Chamaecyparis thyoides* (L.) B.S.P.] were recently excavated by the New Jersey State Highway Department. During the winter of 1947–1948, the Highway Department, in preparation for building a new road, removed great quantities of cedar peat for almost a quarter of a mile on the northeast side of Secaucus. It was the purpose of the excavation to remove the peat down to the clay bottom, at an average depth of ten feet, in order to obtain a sound base for fill material. In the course of the operation, many cedar logs were removed and are now strewn with the peat along the new highway.

Many of these logs are large and well preserved. The largest specimen is shown in figure 1. This tree was three feet d.b.h. when it ceased growing. Unfortunately it had its bole broken at about twenty feet. Its bark is well in place to about ten feet above the base and preserved so that it appears as if the tree had fallen only a short time ago. The bark is not charred, indicating that the tree had not been subjected to any severe burning. Ring counts were made at approximately six feet from the base. This was done by cutting out a section with an axe and smoothing a face with a mallet and

[6] The Oxford, however, says that *litmus*, which came into the language in 1502, is altered from the Old Dutch word that in 1794 gave us *lacmus*, and while referring to the Old Norwegian *litmosi*, does not observe the difference in original meaning. A Norwegian encyclopedia indicates "dye moss" for both derivation and present meaning of the word, and refers to the Old Norse form, *litmosi*. Sigrid Undset mentions the "litmose" as a dye plant in her first volume of "Kristin Lavransdatter."

chisel. There are 304 rings, making the age of the tree at the time of its death 304 years plus the number of years it took for the tree to reach a height of six feet. Korstian and Brush[1] found, from growth studies on white cedar in New Jersey, that under present climatic conditions seven years are required for a tree to grow to six feet. From this information, it then appears that the specimen attained the age of 311 years.

These logs buried below mean sea level at Secaucus are not unusual. Cook noted the occurrence of such logs buried below sea level from Nova Scotia to Georgia as early as 1857.[2] Since this time reports of similarly buried logs have been frequent. With periods of rapid encroachment of the land by the sea, due to land subsidence or rise in sea level or both, the salt-intolerant cedar gradually dies and becomes incorporated with the peat beneath the surface of the coastal marshes.—C. J. Heusser.

Review

The Study of Plant Communities. By Henry J. Oosting. 389 pp. 190 figures. 11 tables. W. H. Freeman & Company, San Francisco, California, 1948.

A good basis for an understanding of general plant ecology is provided in this book. It can be readily understood by the beginning student; yet it is complete enough for a year's course in ecology. The illustrations are well-selected, abundant, but not always printed clearly.

In addition to the introduction, sections on the community, environment, com-

[1] **Korstian, C. F. & Brush, W. D.** 1931. Southern white cedar. U. S. Dep. Agr. Tech. Bull. **251.**

[2] **Cook, G. H.** 1857. On a subsidence of the land on the sea coast of New Jersey and Long Island. Am. Jour. Sci. **74**: 341–354.

munity dynamics and practical aspects are included. In each section the material is well-organized and logically presented. The works of some foreign ecologists as well as those published in North America are surveyed, analyzed, and critically evaluated. The short lists of general references at the ends of chapters, in addition to the extensive bibliography preceding the index, are very useful.

Conopholis americana unfortunately is listed as a saprophyte (p. 27), but it represents one of the few and minor errors.

The last section, "Practical Considerations," should prove interesting to the lay reader. The importance of ecology in the conservation of renewable natural resources and in the affairs of man generally is clearly indicated. Because of this section, it should prove to be an unusually valuable biological textbook.—A. J. Sharp.

Proceedings of the Club

Minutes of the Meeting of December 7, 1948. The meeting was called to order by President Small at 8:05 P.M. at Columbia University; 90 members and friends were present. Dr. B. M. Duggar of the Lederle Laboratories spoke on "The Mycological Background of Aureomycin."

The meeting was adjourned at 9:50; no business was transacted.

Respectfully submitted,
Donald P. Rogers,
Recording Secretary.

INDEX TO AMERICAN BOTANICAL LITERATURE

COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITORS OF THE TAXONOMIC INDEX

TAXONOMY, PHYLOGENY AND FLORISTICS

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